

TRANSGENERATIONAL FECUNDITY COMPENSATION AND
POST-PARASITISM REPRODUCTION BY APHIDS IN RESPONSE
TO THEIR PARASITIDS

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Dedication

To my parents, Marsha A. Kaiser and Charles A. Kaiser.

Abstract

Increased reproductive effort by organisms in response to attack by consumers ('fecundity compensation') is well documented in both plants and animals, though most examples only involve direct compensation by the individuals exposed to consumers. In Chapter 1, I used the parasitoid wasp *Lysiphlebus orientalis* Starý & Rakhshani (Hymenoptera: Braconidae) and the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), to determine whether reproduction by parasitized aphids can lead to fecundity compensation. Although parasitism by *L. orientalis* strongly decreased fecundity for parasitized aphids, offspring of parasitized aphids reproduced at a greater rate at maturity than did the offspring of non-parasitized aphids. Also, parasitized aphids contained fewer but larger embryos developing within them. The presence of these larger embryos may explain how the offspring of parasitized aphids can produce more progeny with no apparent reduction in progeny quality. Mature and nearly mature *A. glycines* successfully reproduced after parasitism, a prerequisite for transgenerational fecundity compensation, and *L. orientalis* showed a preference for these age classes of aphids as hosts when foraging. This work is the first known demonstration of transgenerational fecundity compensation in an animal. In Chapter 2, I demonstrated that *L. orientalis* is able to suppress caged populations of *A. glycines* in spite of transgenerational fecundity compensation by parasitized aphids. Aphid populations exposed to parasitoids were driven to extinction within, on average, 8 or 11 weeks depending on the starting density of parasitoids. I also showed that transgenerational fecundity compensation has a relatively minor impact on modeled *A. glycines* populations. Instead, direct reproduction by parasitized aphids, as well as parasitoid host-stage preference, had stronger impacts. Finally, in Chapter 3, I showed that transgenerational fecundity compensation is not limited to the *A. glycines* – *L. orientalis* association, as it also occurs when *Aphis craccivora* Koch (Hemiptera: Aphididae) is attacked by both *L. orientalis* and *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae). I also found that *L. orientalis* may prefer slightly older *A. craccivora* hosts than *L. fabarum*. These results indicate that while transgenerational fecundity compensation may be an interesting and

novel physiological phenomenon present in multiple aphid-parasitoid associations, it may be relatively inconsequential for populations of aphids and their parasitoids.

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Chapter 1. Parasitoid-induced transgenerational fecundity compensation in an aphid

Introduction

Parasitoids are lethal parasites that often attack immature stages of hosts and prevent reproduction by parasitized individuals (Feener Jr. and Brown 1997, Quicke 1997). As a consequence, discussions of host-parasitoid interactions do not typically address post-parasitism reproduction (Godfray, 1994; Murdoch et al., 2003). However, nymphs of hemimetabolous insects as well as adults of both hemi- and holometabolous insects are also used as hosts by many parasitoids (Spataro and Bernstein 2000, Lin and Ives 2003, Shaw 2004, Johnson et al. 2005, Tepa-Yotto et al. 2013, Maure et al. 2014). Additionally, some parasitoid hosts have been observed to survive and recover following parasitoid development (English-Loeb et al. 1990, Maure et al. 2011). In any of these cases post-parasitism reproduction may occur.

In other animal and plant systems in which reproduction occurs after parasitism or consumption, host individuals often respond by increasing reproduction. Examples include overcompensation in seed set shown by grazed plants (Paige and Whitham 1987, Agrawal 2000) and fecundity compensation by parasitized *Daphnia* spp. (Chadwick and Little 2005, Vale and Little 2012). These compensatory dynamics, along with post-parasitism reproduction more broadly, may be considered examples of non-immune defenses (reviewed by Parker et al., 2011) or extensions of tolerance (reviewed by Baucom & De Roode, 2011). Reproduction by parasitized individuals also allows for maternal effects (transgenerational phenotypic plasticity), where offspring of parasitized individuals may be phenotypically different from offspring of non-parasitized individuals (Mousseau and Fox 1998, Mondor et al. 2005).

Among the hemimetabolous Hemiptera, the diverse and economically important Aphidoidea are often highly phenotypically plastic (Dixon 1998, Srinivasan and Brisson 2012). Parthenogenesis and telescoping generations in aphids make them particularly prone to maternal effects (Mousseau and Dingle 1991). Cues associated with weather, ant mutualists, and natural enemies can induce the production of offspring that differ with respect to color, size, sexual function, the presence of wings, and host-plant preference (Weisser and Stadler 1994, Mondor et al. 2005, 2008, Kunert et al. 2005, Braendle et al. 2006, Hatano et al. 2012). Additionally, some aphids have been shown to exhibit fecundity compensation after being wounded or exposed to pathogens and alarm pheromones (Altincicek et al. 2008, Barribeau et al. 2010, Leventhal et al. 2014). Changes in aphid physiology due to parasitoid eggs, larvae, or venom may also impact developing aphid embryos and be potential mechanisms for maternal effects (Digilio et al. 2000, Falabella et al. 2000, Pennacchio and Mancini 2012).

Here, I use an aphid-parasitoid association to investigate fecundity compensation and maternal effects in the presence of post-parasitism reproduction. To determine effects of parasitism on aphid fecundity, I observed individual aphids and their offspring under laboratory conditions. I expected parasitized aphids to temporarily increase fecundity in response to stinging, similar to what Altincicek et al. (2008) and Leventhal et al. (2014) recorded in response to wounding and some pathogens. To account for any tradeoffs in aphid quality that may be expressed in terms of development time or fecundity of offspring, I also observed individuals from the subsequent generation of aphids. I used dissections to evaluate the effect of parasitism on aphid embryo quantity and size (as a

proxy for quality). Finally, I address the ecological relevance of post-parasitism reproduction in this association by determining the host-stage preference of the parasitoid.

Materials and methods

Insects

I studied the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), an Asian invasive pest of soy, *Glycine max* (L.) Merrill (Fabaceae), in North America (Ragsdale et al. 2011). Soybean aphids reproduce asexually and viviparously on soy under summer conditions, and are primarily wingless. *Lysiphlebus orientalis* Starý & Rakhshani (Hymenoptera: Braconidae) is a recently described, thelytokous parthenogenetic parasitoid wasp collected from soybean aphid's native range in China and held in quarantine as a potential biological control agent of soybean aphid (Starý et al. 2010). *Lysiphlebus orientalis* has also recently been found in Serbia, where it is considered potentially invasive (Petrović et al. 2013). This endoparasitoid is a solitary koinobiont, so the aphid continues to develop temporarily after parasitism and a single aphid individual can only support the development of one parasitoid individual (Godfray 1994).

Soybean aphids were reared in cages on potted soy plants at 23 °C and L16:D8 h photoperiod (see Wyckhuys et al., 2008). Before use in any experiments, aphids were transferred to new potted soy plants with one trifoliate leaf fully expanded to allow for easier identification of developmental stages and to reduce the impacts of crowding.

Lysiphlebus orientalis were from a lab-cultured colony derived from the original collections of the species made in northeastern China in 2006 (Starý et al. 2010). Mummies (parasitoid pupae within an aphid exoskeleton) were transferred from colonies to 1.5-ml microcentrifuge tubes containing small drops of honey. Adult wasps were used in experiments 24-48 h after emergence. As *L. orientalis* is thelytokous (Starý et al. 2010, Petrović et al. 2013), there was no need to track mating status.

Compensation

Single third-instar, fourth-instar, and newly molted (< 24 h) adult apterous aphids were individually transferred with a fine paintbrush from potted source plants to excised inverted soy leaves. First and second instar aphids were not included because preliminary experiments indicated that soybean aphids parasitized at these stages did not reproduce (MC Kaiser, pers. obs.). Aphids were allowed to settle for 5 min and then one newly emerged adult female *L. orientalis* was introduced near an aphid using a fine paintbrush, and both were covered with a 6-mm-wide clear plastic observation dome. After a successful ‘sting’ was observed under a dissection microscope (described below, shown in Figure 1, and similar to Tentelier et al., 2006), the wasp was removed and the aphid was transferred to a singly-potted soy plant with unifoliate leaves just beginning to unfold. A layer of sand was added above the soil to reduce soil-dwelling Diptera. A 355-ml clear plastic cup with the bottom removed was placed over the plant and pressed into the sand. The top was then covered with no-see-um mesh secured by a rubber band to ensure aphid containment. Eight third-instar, 11 fourth-instar, and 12 adult aphids were

allowed to be stung. As a control group for comparison, an additional 10 third-instar, 10 fourth-instar, and 14 adult aphids were similarly handled but not exposed to parasitoids. Stung aphids that failed to mummify (i.e., a single aphid) plus offspring were removed from the experiment and not included in the replicates.

Aphid stage, reproductive state, and fecundity were observed daily until aphids mummified. The 1st day that new nymphs were observed, a single nymph from this cohort was transferred to a new plant for daily observations. This second generation included 11 and 10 aphids whose parents were stung or not stung in the fourth stadium, and 12 and 14 aphids whose parents were stung or not stung as adults, respectively. The 1st day that a nymph from this cohort reached maturity and began producing nymphs of its own, one of these nymphs was transferred to a new plant for daily observations. This third generation included 10 and seven aphids whose grandparents were stung or not stung in the fourth stadium, as well as 11 aphids for both cases where grandparents were stung or not stung in the adult stage. A total of eight aphids from the third generation were lost, which is reflected in the replicates. I continued to observe each of these three generations of aphids from the two lineages for at least 4 days after reproductive maturity. All aphids were housed in the same climate-controlled rearing room, with treatments and generations interspersed across bench space and time. I compared the numbers of nymphs produced by aphids in both lineages each day, with the developmental stage and stung status of the original parent as factors in two-way ANOVA and with P-values adjusted using Bonferroni corrections in R v. 3.0.0 (R Core Team 2013). I also pooled reproduction across the 4 days to determine total effect of the

two factors on nymph production. Residuals were examined to ensure that model assumptions were met.

Aphid dissections

I allowed individual fourth-instar aphids to be stung by newly emerged adult female *L. orientalis* wasps as described above. Additional fourth-instar aphids from the same populations were similarly handled but not exposed to parasitoids as a control group. Aphids were then transferred to soy plants with one fully expanded trifoliate leaf. One, 3, or 5 days after being stung, aphids were dissected under a dissecting microscope on glass slides in 15 μ l drops of insect Ringer's solution (0.75 g NaCl, 0.035 g KCl, and 0.02 g CaCl_2 in 100 ml distilled H_2O) using size 0 insect pins and fine forceps. The number of visible and intact embryos was recorded without adding a coverslip. Twelve aphids from both 'stung' and 'not stung' treatments were dissected at each time interval, resulting in 72 total dissections. For aphids dissected 3 days after being stung, the length and width of the largest embryo (next in birth order) was recorded using an ocular micrometer. Embryo volume was estimated by applying the length (l) and width ($2r$) measurements to the volume of a cylinder ($\text{volume} = \pi r^2 l$). Embryo size and number were compared between aphids stung or not stung using two-tailed t-tests in R (R Core Team 2013). Aphid birth weight was not measured due to the logistical difficulty of preventing feeding by nymphs after birth, which could mask effects of the maternal environment.

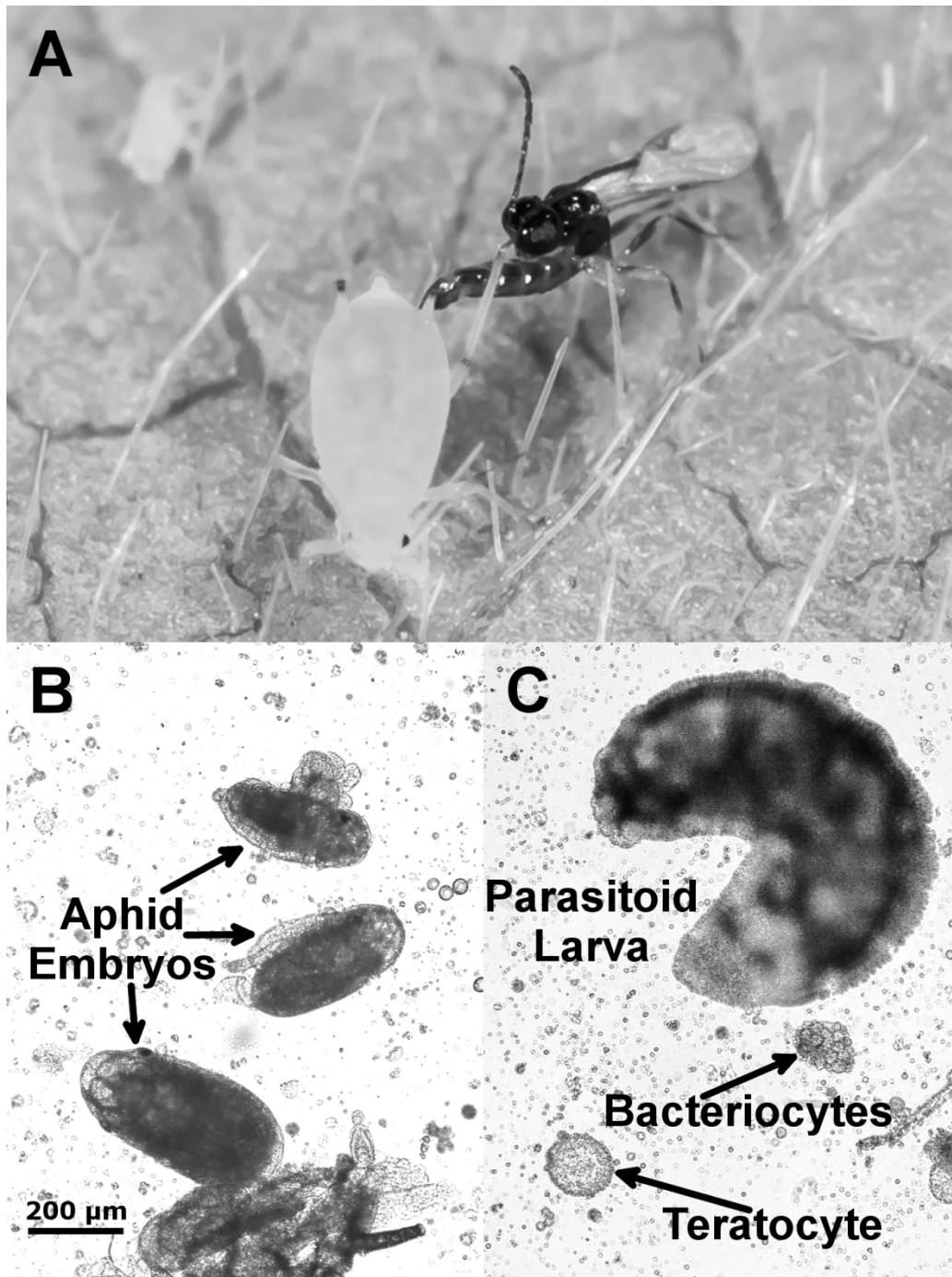


Figure 1. Organisms and dissections. (A) Adult female *Lysiphlebus orientalis* stinging a fourth-instar soybean aphid (photo: MC Kaiser). (B) Aphid embryos from an adult aphid. (C) Parasitoid larva, teratocyte, and bacteriocytes (aphid cells containing nutritional endosymbionts) 7 days after parasitism. The aphid has ceased reproduction by this time as all embryos have been destroyed, and the parasitoid is preparing to pupate. Scale same as for (B).

Preference

I conducted choice tests of parasitoid preference for aphid stage in a manner similar to Wyckhuys et al. (2008) for a different parasitoid of the soybean aphid. Newly emerged adult female *L. orientalis* were presented with 25 apterous aphids (five each in the first, second, third, and fourth stadium, and adults), which were allowed to settle for 5 min on an inverted soy leaf prior to introduction of the wasp. I monitored wasp behavior by recording ‘encounters,’ ‘attacks,’ and ‘stings’ over a 5-min period. Encounters were defined as any physical contact of an aphid made by the wasp. Attacks consisted of behavior where the wasp bent its abdomen towards an aphid, including making contact with its ovipositor. Stings consisted of the final extension of the wasp’s abdomen typically indicative of successful oviposition. Although observed stings do not guarantee oviposition or parasitism, only one aphid observed as stung in the compensation experiment described above failed to mummify. I assessed a total of 11 parasitoid individuals, with leaves and aphids being replaced prior to each assessment. Aphid defensive behaviors were not scored, though Wyckhuys et al. (2008) found them to be stronger in later stages of *A. glycines*.

Analysis of preference was modified from Weisser (1994) and Wyckhuys et al. (2008), using Manly’s beta statistic (Manly 1974). Preference was scored as the deviation in the number of individual aphids stung from the number of aphids encountered by calculating

$$\beta_j = \frac{(x_j/A_j)}{\sum_{i=1}^5 x_i/A_i},$$

where x_i is the number of stings and A_i is the total number of encounters recorded for aphids in growth stage i , and x_j is the number of stings and A_j is the total number of encounters for aphids in growth stage j , with five growth stages considered (i.e., $j = 1, 2, 3, 4, 5$). If β_j is equal to $1/5$ for all stages, the parasitoid shows no preference, but if β_j is significantly greater than $1/5$ for any given stage, the parasitoid prefers that stage. I chose this formulation of Manly's beta, which allows for repeated behaviors, because I observed parasitoids encountering and stinging individual aphids multiple times in the same trial. I compared Manly's beta values using a non-parametric Kruskal-Wallis test, followed by a Nemenyi pairwise test for multiple comparisons of mean rank sums from the PMCMR package in R (R Core Team 2013, Pohlert 2014).

Results

Compensation

No aphids stung in the third stadium became reproductive, but fourth-instar and adult soybean aphids stung by *L. orientalis* successfully reproduced for up to 4 days (Figure 2). Parasitism significantly reduced aphid fecundity, primarily due to a significant decrease on day 4 (ANOVA, all days pooled: $F_{1,43} = 15.133$, $P < 0.001$; day 1: $F_{1,43} = 0.003$, corrected $P = 1.0$; day 2: $F_{1,43} = 0.760$, corrected $P = 1.0$; day 3: $F_{1,43} = 6.602$, corrected $P = 0.055$; day 4: $F_{1,43} = 73.23$, corrected $P < 0.001$). There was no significant effect of aphid stage on overall reproduction over the first 4 days for these aphids (all days pooled: $F_{1,43} = 0.998$, $P = 0.32$; days 1-4 separately: corrected $P > 0.4$). Among the next generation of aphids, there was also no significant effect of the mother's stage when

stung (all days pooled: $F_{1,43} = 0.358$, $P = 0.55$; each day 1-4 separately: corrected $P = 1.0$). However, there was a significant effect of mothers' parasitism on reproduction (all days pooled: $F_{1,43} = 5.424$, $P = 0.025$), with offspring of parasitized aphids reproducing at a greater rate than offspring of not stung aphids (Figure 3A). The increase was equal to approximately one additional nymph produced per day by the offspring of parasitized compared to non-parasitized aphids, though there were no significant differences between treatments by day once P-values were corrected (day 1: $F_{1,43} = 0.842$, corrected $P = 1.0$; day 2: $F_{1,43} = 3.351$, corrected $P = 0.30$; day 3: $F_{1,43} = 4.155$, corrected $P = 0.19$; day 4: $F_{1,43} = 4.282$, corrected $P = 0.18$). Second-generation progeny of parasitized aphids reproduced at the same rate over the first 4 days of reproduction as those of non-parasitized aphids (Figure 3B; all days pooled: $F_{1,35} = 0.031$, $P = 0.86$). There was also no effect of the stage of grandmothers who received treatment (all days pooled: $F_{1,35} = 1.428$, $P = 0.24$). Overall reproduction increased slightly with each generation, which may be attributed to successive generations being raised under less crowded conditions than the source colonies.

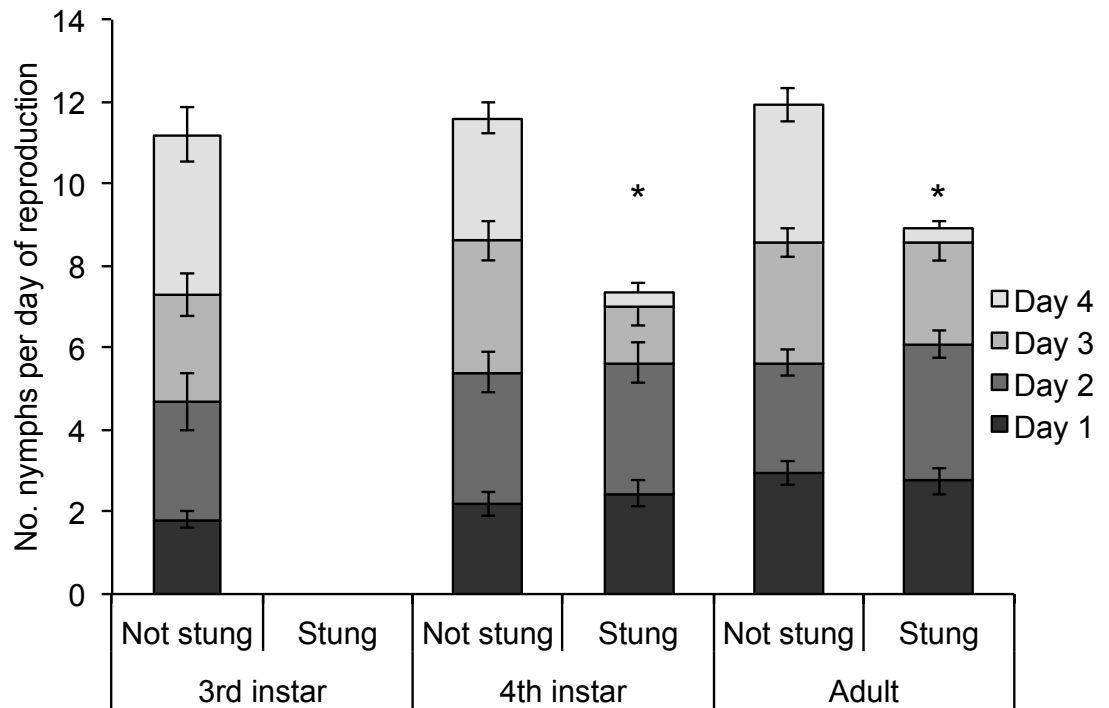


Figure 2. Mean (\pm SEM) daily reproduction by *Aphis glycines* aphids after being stung or not stung by *Lysiphlebus orientalis* as third or fourth instar, or as newly molted adult. ‘Days’ are days of reproduction, with ‘day 1’ being the first day nymphs were observed. Each bar represents the mean number of nymphs laid, separated by day. Asterisks indicate significant effect of parasitism on pooled reproduction (two-way ANOVA: $P < 0.05$).

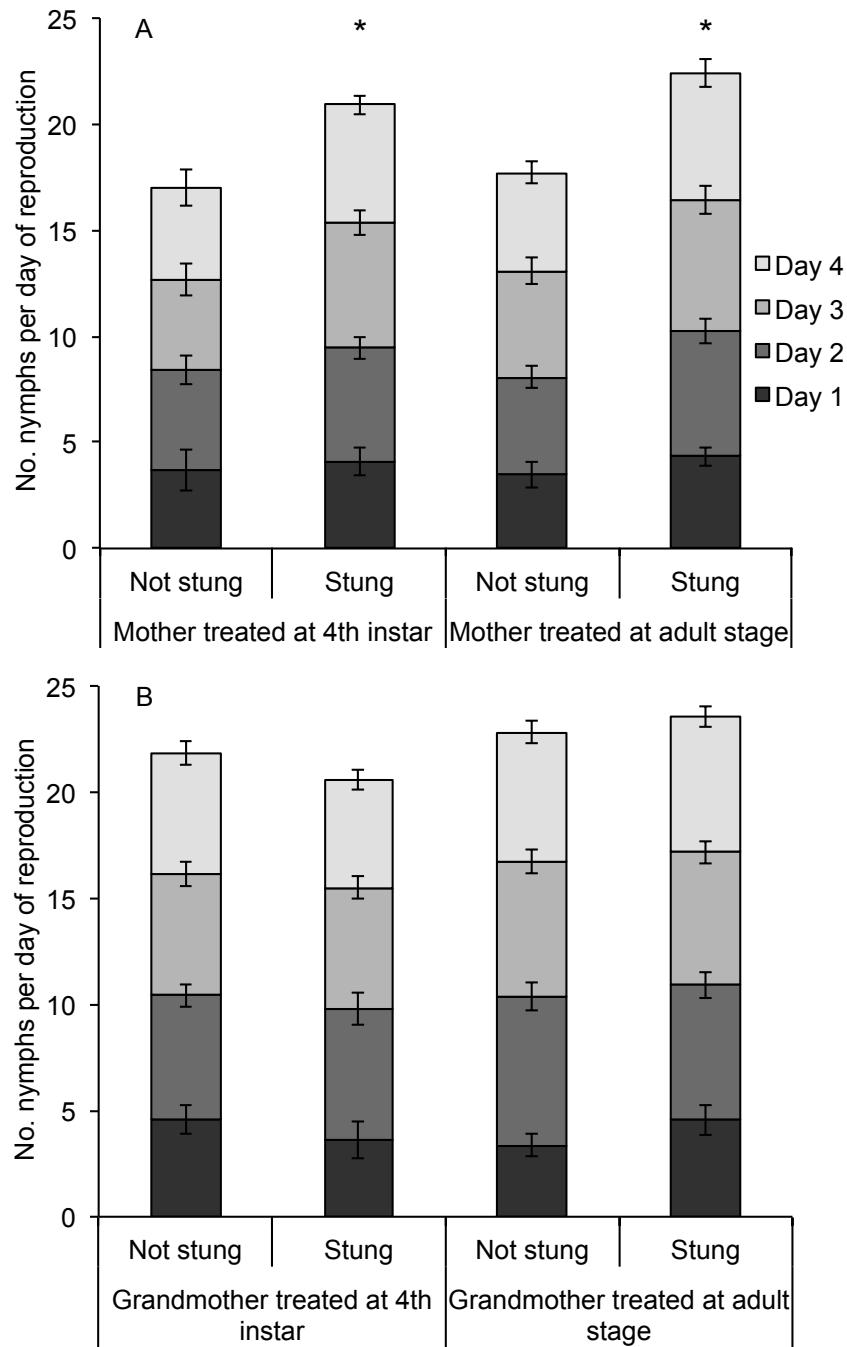


Figure 3. Mean (\pm SEM) daily fecundity of *Aphis glycines* aphids whose (A) mothers or (B) grandmothers were either stung or not stung by *Lysiphlebus orientalis* as fourth instar or adult. ‘Days’ are days of reproduction, with ‘day 1’ being the first day nymphs were observed. Each bar represents the mean number of nymphs laid, separated by day. Asterisks indicate significant effect of mothers’ parasitism on pooled reproduction (two-way ANOVA: $P < 0.05$).

Aphid dissections

There was no significant effect of parasitism on embryo number in aphids 1 day after being stung (Figure 4; $t = 1.26$, d.f. = 22, $P = 0.22$). However, 3 days after being parasitized, aphids had significantly fewer embryos than non-parasitized aphids ($t = 9.03$, d.f. = 22, $P < 0.001$). Only two aphids still contained a single healthy looking embryo 5 days after being stung, so the distribution of data was far from normal and not suitable for a parametric test (Figure 4). This result is consistent with the observation from the compensation experiment above that parasitized adult aphids never successfully reproduced more than 4 days after being parasitized. When aphids were dissected 3 days after parasitism, the largest embryo (which was the next in birth order within the ovaries) was significantly larger than the embryos from aphids that were not parasitized (stung: mean \pm SEM = $7.56 \pm 0.391 \times 10^6 \mu\text{m}^3$; not stung: $5.49 \pm 0.369 \times 10^6 \mu\text{m}^3$; $t = 3.85$, d.f. = 22, $P = 0.001$).

Preference

Aphid growth stage had a significant effect on oviposition preference by adult female *L. orientalis* (Kruskal-Wallis: $\chi^2 = 11.94$, d.f. = 4, $P = 0.018$), and there were significant pairwise differences in preference between adult aphids and first-instar ($P = 0.047$) and second-instar nymphs ($P = 0.050$). Though all aphid stages encountered were accepted as hosts, older hosts were preferred (Figure 5).

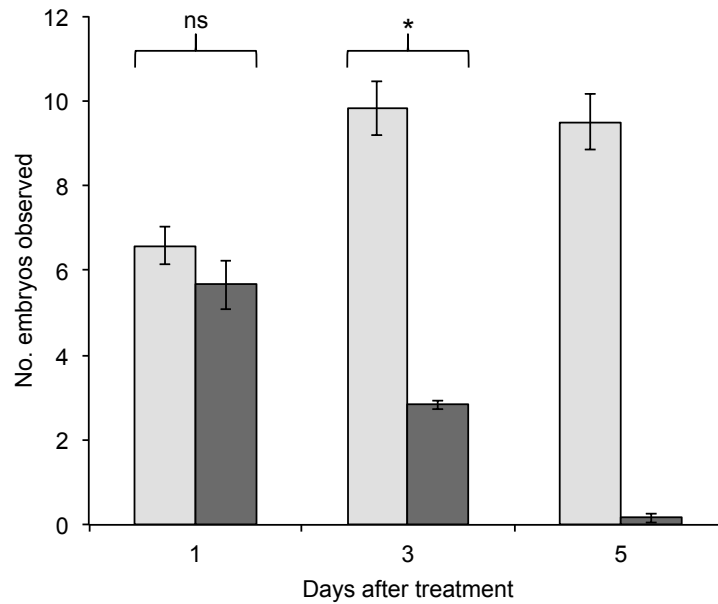


Figure 4. Mean (\pm SEM) number of embryos inside *Aphis glycines* aphids 1, 3, or 5 days after being stung (dark bars) or not stung (light bars) by *Lysiphlebus orientalis* in the fourth stadium. One day after treatment, there was no significant difference (n.s.) between stung and not stung aphids, whereas 3 days after treatment, stung aphids contained significantly fewer (*) intact embryos (two-tailed t-test: $P < 0.05$). Only two aphids contained a single healthy looking embryo 5 days after being stung.

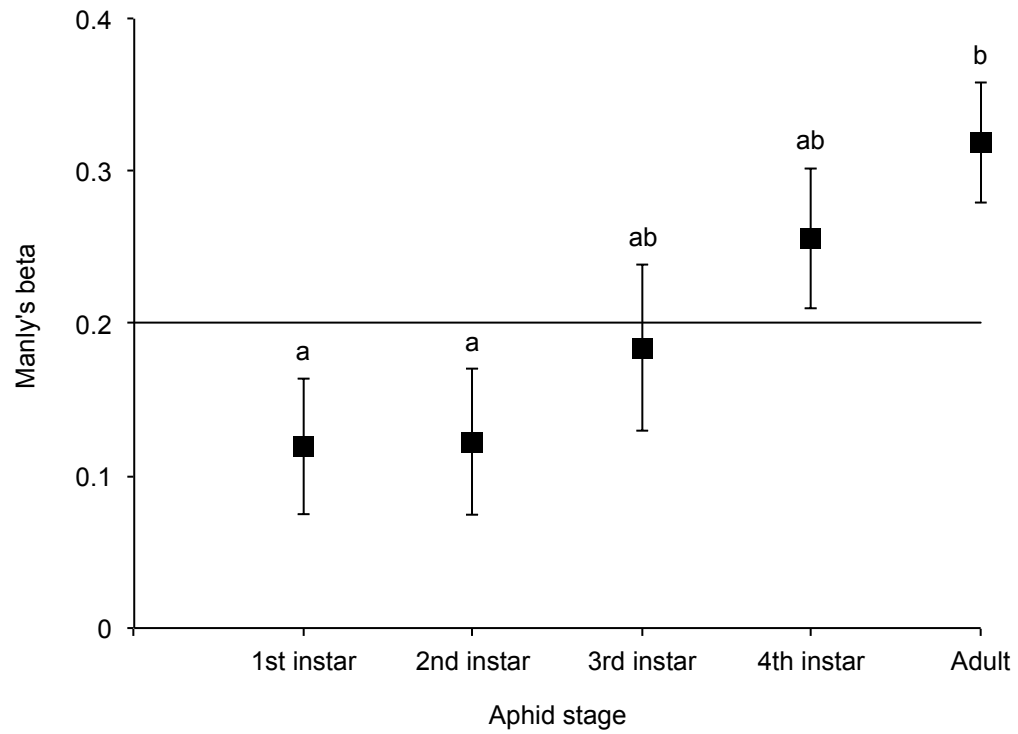


Figure 5. Mean (\pm SEM) host stage preference of *Lysiphlebus orientalis* given encounter rate. The horizontal line corresponds to Manly's β value (0.2) for no preference between stages. Values above the line suggest preference. Means capped with different letters are significantly different (Nemenyi pairwise comparisons: $P < 0.05$).

Discussion

I did not find evidence for fecundity compensation by parasitized aphids, as they produced no more offspring than did non-parasitized aphids during any 24-h interval. However, the offspring of parasitized aphids reproduced at a significantly greater rate than the offspring of non-parasitized aphids once they reached maturity. Although I suspected a tradeoff between the number and quality of nymphs in this generation, this did not appear to be the case, as the following generation in parasitized lineages reproduced at the same rate as those from non-parasitized lineages. Transgenerational fecundity compensation has, to our knowledge, never before been reported in the literature. Despite this phenomenon being restricted to the offspring of parasitized fourth-instar and adult aphids, it is still likely to be ecologically relevant, as these stages are readily accepted as hosts and even preferred by *L. orientalis* females. The acceptance of, or preference for, late-stage hosts has been reported for other aphid parasitoids (Völkl et al. 1990, Lin and Ives 2003, Barrette et al. 2010, Hopkinson et al. 2013), but is not typical and may have various effects on parasitoid development (Hågvar and Hofsvang 1991, Sequeira and Mackauer 1992, Barrette et al. 2009).

Dissections of parasitized aphids shed some light on the possible mechanisms for the transgenerational fecundity compensation that I documented. As the embryos measured for size were the next in birth order, they likely would have been born within hours at or near that size. These estimates of body volume should therefore be correlated with birth weight, which Traicevski & Ward (1994) showed to be linked with fitness at adulthood in the cowpea aphid, *Aphis craccivora* Koch. However, it is still not clear

whether the increase in embryo size I observed constitutes an adaptive investment in high-quality offspring by dying mother aphids, the result of physiological manipulation by parasitoids, or simply a side effect of parasitism. Regardless of mechanism, however, there is a change in phenotype induced by a change in the maternal environment.

Soon after parasitism, parasitoid venom induces apoptosis in aphid oocytes, preventing new embryos from forming (Digilio et al. 2000, Falabella et al. 2007, Pennacchio and Mancini 2012). This may lead to reduced competition for resources among the surviving embryos prior to parasitoid egg hatch. Furthermore, teratocytes (free cells originating from an extraembryonic membrane in some parasitoids) have been shown to increase activity of aphid nutritional endosymbionts before facilitating the breakdown of remaining aphid embryos and tissues (Tremblay and Caltagirone 1973, Dahlman and Vinson 1993, Falabella et al. 2000, 2005, Cloutier and Douglas 2003, Pennacchio and Mancini 2012). This action could further enrich the aphid embryonic environment in the early stages of parasitoid larva development. Interestingly, Baverstock et al. (2006) showed that there is no next-generation reproduction compensation in pea aphids (*Acyrtosiphon pisum* Harris) whose parents are infected by entomopathogenic fungi. Although I did not directly measure aphid stress, this suggests transgenerational fecundity compensation is not a general stress response by challenged aphids but is instead the result of reduced competition between aphid embryos due to parasitoid venom and teratocytes. It remains to be shown whether this effect can be generalized to other parasitoid and aphid species, or to other organisms beyond aphids.

Aphid-parasitoid associations, and other interactions where post-parasitism

reproduction occurs, provide unique opportunities to consider the role of fecundity compensation in population dynamics. Population-level consequences of the transgenerational compensation reported here have yet to be determined. However, *L. orientalis* was precluded from release as a biological control agent against soybean aphid because of this compensation despite having a relatively narrow host range and appearing to be an otherwise promising candidate (Z Sezen, J Dregni & GE Heimpel, unpubl.). Though limited reproduction by parasitized soybean aphids has been thought to have little impact on modeled populations (Lin and Ives 2003), any associated maternal effects such as those observed here may increase this impact. Spataro & Bernstein (2000) used an age-structured model to show that reproduction by parasitized hosts in any system can lead to dampened cyclic dynamics or even prevent host population regulation. The compensation presented in our work could exaggerate the outcomes highlighted by these authors and, if found to affect population dynamics, could be added to the lists of traits of potential biological control agents that attack plants or adult stages of insects to be examined before use (Hopper 2001, Pearson and Callaway 2003, Heimpel et al. 2004, Kidd and Jervis 2005, Babendreier et al. 2006, Messing et al. 2006, Myers 2007).

Compensation by hosts could also reduce non-target effects of biological control agents and pests. Johnson et al. (2005) showed that non-target effects of tachinid biological control agents on Hawaii's endemic koa bug, *Coleotichus blackburniae* White, can be strong at certain locations of high host density. Though these researchers stated that parasitized adult koa bugs have reduced reproductive output, the effect is less pronounced than the more typical effect of parasitism, which is death of the immature

host. In this case it is not clear whether any form of fecundity compensation could be ameliorating non-target impacts. In a different association, herbivore-induced compensation in potatoes (*Solanum tuberosum* L.) in response to light to moderate infestation of the Guatemalan tuber moth, *Tecia solanivora* Povolný, has been demonstrated to increase crop yield (Poveda et al. 2010, 2012). The mechanisms in this case and others of herbivore-mediated compensation resulting in mutualism in plants restrict compensation to the individuals being attacked and may not allow for transgenerational fecundity compensation (Agrawal 2000, Poveda et al. 2010, 2012).

Of further interest is whether other combinations of aphid and parasitoid species produce the same transgenerational fecundity compensation, given both their ecological and agricultural importance. Fecundity compensation in response to microbial and physical challenges has been well documented in pea aphid (Altincicek et al. 2008, Barribeau et al. 2010, Leventhal et al. 2014). In these cases, short-term increased reproduction occurs at the expense of decreased fecundity later in life, and is not associated with a tradeoff in offspring quality (Leventhal et al. 2014). Again, however, the fecundity compensation discussed by these authors occurs in the individuals being challenged, not their offspring. Whereas the research on *L. orientalis* presented here was conducted in quarantine in the USA, *L. orientalis* was recently detected in northern Serbia (Petrović et al. 2013). There, it is believed to be increasing in numbers and expanding its host range, and may be competing with the native congener *Lysiphlebus fabarum* (Marshall), an important biological control agent in the region (Petrović et al. 2013). Post-parasitism reproduction and compensatory reproduction by hosts of *L.*

orientalis may increase its ability to establish in new locations if they lead to diminished suppression of host populations. It is not known whether *L. fabarum* or any other aphid parasitoids induce the same response in their hosts.

Aphids that reproduce after successful parasitoid attack should see a selective advantage over those that cannot, and this advantage should be even greater if their offspring have increased fecundity. Leventhal et al. (2014) showed that compensation by hosts should occur in associations where virulence of the parasite is high and where either some hosts survive attack or cues associated with the threat of attack are available. These conditions are met in our study system, as parasitized aphids can both reproduce and alert conspecifics to the threat of attack using alarm pheromones (see Vandermoten et al., 2012).

Host compensation may also be adaptive for the parasitoid. Modeling by Dubois et al. (2013) suggests that parasite-induced compensation is most likely to occur in non-trophically transmitted parasites, such as parasitoids. As outlined above, physiological manipulations of the host by parasitoids and their teratocytes may be responsible for the mechanisms underlying transgenerational fecundity compensation in our system.

Although I would not classify the interaction between soybean aphid and *L. orientalis* as mutualistic, the compensation occurring may be beneficial to both the host and parasite relative to aphid-parasitoid systems without fecundity compensation.

Studies of maternal effects in viviparous organisms have historically focused on mammals (Bernardo 1996). More recently, however, aphids have proven to be excellent systems for investigating maternal effects (Kunert et al., 2005; Mondor et al., 2005, 2008;

Hatano et al., 2012). Natural enemy-induced maternal effects, particularly those that affect population growth or structure, may have substantial impacts on host ecology in the context of biological control. Continued documentation and interpretation of these effects will be useful for assessing introduced organisms such as biological control agents and invasive species, and making predictions of establishment, population growth, and host suppression for species not yet introduced.

Chapter 2. Transgenerational fecundity compensation in *Aphis glycines* does not prevent suppression by *Lysiphlebus orientalis* and has minimal effects on aphid populations

Introduction

Parasitoid insects (obligatory lethal parasites) have both fascinated and perplexed humans for centuries, and were first accurately described in China over 900 years ago (Cai et al. 2005, van Lenteren 2005). These insects often suppress host populations (Murdoch et al. 1985, 2005) and this has led to their frequent use as biological control agents (Hågvar and Hofsvang 1991, Boivin et al. 2012, van Lenteren 2012). Before this can be done however, they must be deemed safe and potentially effective (Howarth 1991, Pearson and Callaway 2003, Barratt et al. 2010, 2016). This work is usually done in laboratory trials under quarantine conditions if the parasitoid is imported, and may be assisted with the use of models.

Laboratory micro- and mesocosm studies have been used to help better understand parasitoid-host population dynamics for decades (see Hassell and Waage 1984, Hassell 2000). For example, Utida (1950, 1957) used laboratory populations sustained over multiple generations to demonstrate mathematically predicted cyclic interactions between parasitoids and their hosts. Caged populations have also been used to determine the effects of environmental temperature on host and parasitoid persistence (Tuda and Shimada 1995). Bonsall and Hassell (1997, 1998) used caged populations to show how apparent competition can cause extinction in host populations. While the dynamics observed in cages may not be exactly manifested in the field, laboratory studies allow for greater environmental control and the ability to make predictions about the dynamics of introduced species before they are present in an environment.

My work focuses on one host-parasitoid system where, in Chapter 1, I have shown that offspring of parasitized hosts (*Aphis glycines*) reproduce at a greater rate than offspring of non-parasitized hosts (transgenerational fecundity compensation). Also, the parasitoid (*Lysiphlebus orientalis*) preferentially attacks older hosts, which are mature enough to reproduce before being overcome by the developing parasitoid larva. At the population level, this combination of parasitoid preference and host compensation may allow the host to maintain high numbers despite high parasitism rates.

While transgenerational fecundity compensation has not been described prior to this work, direct fecundity compensation by hosts in response to parasitic organisms has been demonstrated in other systems (e.g. Barribeau et al. 2010, Vale and Little 2012, Hendry et al. 2016) and should benefit host populations. Multi-generation, population-level effects of these cases have not been explored. Individual-based compensation mechanisms are not the only ways which compensation may occur however. Mortality alone in stage-structured populations has been found to have positive effects on population growth in several systems, such as fish (Ohlberger et al. 2011) and invasive plants (Pardini et al. 2009). This so-called ‘hydra effect,’ where increased mortality leads to increased growth (reviewed by Abrams (2009) and Schröder et al. (2014)), is becoming more widely appreciated and may provide yet another means for host suppression to be thwarted.

A major question raised by the work in Chapter 1 is whether transgenerational fecundity compensation could disrupt aphid population suppression by parasitoids. To answer this question, I used a two-pronged approach. First, caged populations of aphids

were established in quarantine and either exposed or not exposed to parasitoids. I hypothesized that populations of aphids alone would persist and stabilize over time, while those exposed to parasitoids would eventually be driven to extinction. I also hypothesized that populations exposed to higher initial densities of parasitoids would be driven to extinction sooner. If aphid populations exposed to parasitoids persisted, then fecundity compensation by offspring of parasitized aphids may be responsible. Second, a two-stage matrix model representing a population of *A. glycines* under parasitoid attack was constructed. Several parameters were varied to determine effects of post-parasitism reproduction, transgenerational fecundity compensation, and parasitoid host-stage preference on the threshold parasitism pressure necessary to prevent aphid population growth. If any factor, but especially transgenerational fecundity compensation, is important for aphid population growth, then removing it from the model should result in a large change in threshold parasitism pressure.

Methods

Insects

As in Chapter 1, *Aphis glycines* were reared in cages on potted soy plants at 23 °C and L16:D8 h photoperiod. *Lysiphlebus orientalis* were from the same lab-cultured colony derived from the original collections of the species made in northeastern China in 2006 (Starý et al. 2010). Parasitoid mummies were transferred from colonies to 1.5-ml microcentrifuge tubes containing small drops of honey prior to populating cages. Adult female wasps were used <24 h after emergence. Since *L. orientalis* reproduces asexually

and males did not exist in the source colony, virgin wasps were used.

Population cages

Cages were 30 cm x 40 cm x 40 cm clear plexiglass with 10 cm diameter circular mesh covered holes on four sides. All cages were started with six individually potted soy plants with one trifoliate leaf fully expanded. Each plant was seeded with 50 soybean aphids of evenly mixed instars taken from small leaf and stem cuttings of infested source plants. Cages were then assigned to one of three treatment groups: Low, High, and Control. Cages with low initial parasitoid density received 3 newly emerged (<24 hours old) adult female wasps, while cages with high starting parasitoid density received 30 newly emerged adult female wasps. Control cages did not receive any parasitoids. Starting ratios of parasitoids to aphids were therefore 1:100 and 1:10 for 'low' and 'high' treatments respectively. Eight cages of each treatment were established for a total of 24 cages. Treatments and replicates were mixed haphazardly through bench space and time within a single climate-controlled room. Cages were watered as needed to keep soil moist but to minimize excess humidity within the cage. Four small desktop fans were placed on the benches in the room to keep air circulating through the cages.

For the following 13 weeks (or until insect populations went extinct), every 3 or 4 days, one plant was removed from each cage, cut at the base of the stem, and all aphids and aphid mummies (pupal-stage parasitoids) were counted. One new uninfested plant, onto which the cut plant stem and all insects were draped, was then added to the cage. Additional observations of each collected plant included the number of emerged

mummies, the number of trifoliate leaves of the plant, the number and proportion of winged (alate) aphids, and the proportion of fourth-instar plus adult aphids of the population (those capable of reproducing after parasitism, rounded to the nearest 0.10). Insect counts were made for one cage at a time under an insect containment hood, with surfaces wiped down with 70% EtOH and paper towel, and hands washed between cages. Control cages were always handled first on any given day to further reduce the likelihood of parasitoid contamination.

Cumulative aphid days (CAD) were calculated for each cage over time as a representation of aphid pressure on plants by determining

$$\sum_{n=1}^n \left(\frac{(a_n + a_{n+1}) \times 3.5}{2} \right)$$

where a_n equals the number aphids counted at sampling date n with sampling dates occurring in sequential observations that were on average 3.5 days apart, based on the insect-days method developed by Ruppel (1983) and used by Chacón et al. (2012). Differences in CAD between either treatment containing parasitoids and the aphid-only treatment were evaluated for each observation interval n using two-tailed t-tests in R (R Core Team 2013).

Model

A stage-structured Leslie matrix model (see Leslie 1945) was constructed to simulate a population of *A. glycines* under attack by *L. orientalis* (Table 1), similar to that developed by Lin and Ives (2003) for *A. glycines* parasitized by *Aphidius colemani* (Viereck). Two

matrices were used in order to account for transgenerational fecundity compensation by the offspring of parasitized aphids. In the first matrix, stages $S_1 - S_4$ represent 1st – 4th instar nymph aphids. S_5 represents pre-reproductive adults (aphids that have molted to adulthood but not yet begun reproducing), while A represents reproductive adults. Stages P_1 through P_3 are parasitized mature aphids. Each column reads as the probabilities of transition from that stage at time t to the row's stage at time $t + 1$ (where t is measured in days). Transition probabilities between stages S_i and A represent mean values of daily observations of non-parasitized individual soybean aphids in Chapter 1. Mortality due to parasitism for each stage is accounted for by p_i . Here, $p_i = \exp(a_i y)$, where a_i is the stinging preference for aphids in the five stages i as shown in Figure 5 ($a_1 = 0.12$, $a_2 = 0.12$, $a_3 = 0.18$, $a_4 = 0.26$, $a_5 = 0.32$) and y is the overall scaling term for parasitism pressure. Daily nymph mortality is not explicitly represented in Table 1, but can be added by multiplying all nymph stage transition probabilities by a fixed survival proportion,. Daily adult survival is fixed at 0.86 and is based on observations of adult *A. glycines* in colonies by Lin and Ives (2003). Daily reproduction by adult *A. glycines* is based on observations from Chapter 1 and equals 2.89 nymphs per day, similar to the value of 2.56 nymphs per day reported by Lin and Ives (2003). Reproduction by parasitized aphids continues for three days, decreasing to 1.97 nymphs on the third day. The second matrix mirrors the first, with a lowercase “a” added to the stage names (such as Sa_1 , Aa , Pa_1 , etc.).

Table 1. Stage-structured matrix model of *Aphis glycines* population growth when under parasitoid attack.

Values in row i and column j give the probability of an individual transitioning from stage j at time t to stage i at time $t + 1$, when $t = 1$ day. S_1 to S_4 represent 1st – 4th instar nymph aphids. S_5 represents pre-reproductive adult aphids, and A represents reproductive adult aphids. Stages P_1 through P_3 are parasitized mature aphids. The top matrix represents a traditional aphid-parasitoid interaction, while the second accounts for transgenerational fecundity compensation. Underlined values represent transitions between the two matrices. Values to the right of the dashed line account for post-parasitism reproduction.

| | S_1 | S_2 | S_3 | S_4 | S_5 | A | | P_1 | P_2 | P_3 |
|--------|-----------|-----------|-----------|-----------|-----------|--|--|---|---|---|
| S_1 | $0.39p_1$ | | | | | $2.89 \rightarrow S_1$ | | <u>$2.89 \rightarrow Sa_1$</u> | <u>$2.89 \rightarrow Sa_1$</u> | <u>$1.97 \rightarrow Sa_1$</u> |
| S_2 | $0.59p_1$ | $0.37p_2$ | | | | | | | | |
| S_3 | $0.02p_1$ | $0.61p_2$ | $0.30p_3$ | | | | | | | |
| S_4 | | $0.02p_2$ | $0.67p_3$ | $0.40p_4$ | | | | | | |
| S_5 | | | $0.03p_3$ | $0.13p_4$ | | | | | | |
| A | | | | $0.47p_4$ | p_5 | $0.86p_5$ | | | | |
| P_1 | | | | $1 - p_4$ | $1 - p_5$ | $0.86p_5(1 - p_5)$ | | | | |
| P_2 | | | | | | | | 0.86 | | |
| P_3 | | | | | | | | | 0.86 | |
| | Sa_1 | Sa_2 | Sa_3 | Sa_4 | Sa_5 | Aa | | Pa_1 | Pa_2 | Pa_3 |
| Sa_1 | $0.39p_1$ | | | | | <u>$3.41 \rightarrow S_1$</u> | | $3.41 \rightarrow Sa_1$ | $3.41 \rightarrow Sa_1$ | $2.32 \rightarrow Sa_1$ |
| Sa_2 | $0.59p_1$ | $0.37p_2$ | | | | | | | | |
| Sa_3 | $0.02p_1$ | $0.61p_2$ | $0.30p_3$ | | | | | | | |
| Sa_4 | | $0.02p_2$ | $0.67p_3$ | $0.40p_4$ | | | | | | |
| Sa_5 | | | $0.03p_3$ | $0.13p_4$ | | | | | | |
| Aa | | | | $0.47p_4$ | p_5 | $0.86p_5$ | | | | |
| Pa_1 | | | | $1 - p_4$ | $1 - p_5$ | $0.86p_5(1 - p_5)$ | | | | |
| Pa_2 | | | | | | | | 0.86 | | |
| Pa_3 | | | | | | | | | 0.86 | |

Transitions between the two matrices are underlined, where parasitized stages P_1 through P_3 produce Sa_1 nymphs while non-parasitized Aa adults produce S_1 nymphs. The main feature of the second matrix is greater reproduction by adult aphids, which was determined by multiplying the percent increase observed in Figure 3A by the rate in the first matrix. Reproduction by parasitized adults in the second matrix, $Pa_1 - Pa_3$, is the same as for Aa for two days, then decrease by the same proportion as the decrease reported for P_3 in the first matrix. Stages $Pa_1 - Pa_3$ produce ‘regular’ S_1 nymphs because no difference in reproduction was seen for aphids whose grandmothers were or were not parasitized (Figure 3B).

Model parameters were manipulated to determine the threshold parasitism pressure, y , and corresponding percent parasitism ($= 20y$) necessary to prevent aphid population growth under several scenarios. The first scenario reflected ‘host castration,’ where parasitized aphids are unable to reproduce (Baudoin 1975). This includes only stages from the upper matrix, left of the dashed line in Table 1. The second scenario allowed for post-parasitism reproduction but no transgenerational fecundity compensation, so only used the top matrix. The third scenario included both post-parasitism reproduction and transgenerational fecundity compensation so includes all of both matrices in Table 1, and is the closest representation of the interaction between *A. glycines* and *L. orientalis*. The fourth and final scenario was the same as the third except that the parasitoid stage preference was changed such that all $a_i = 0.2$, reflecting a parasitoid that shows no preference for specific aphid stages. To determine the relative sensitivity of the model to aphid survival, all four scenarios were run when background

daily nymph survival was equal to both 0.75 and 0.8. Aphid density over 50 days was examined for the four scenarios outlined above when parasitism pressure was equal to 20% and 50% parasitism. Additionally, aphid intrinsic growth rate, r , was estimated at day 50 by calculating $r = \ln (N_{t=50} / N_{t=49})$, population growth was stable by this point for all scenarios presented above. Finally, age structure of these modeled populations was also examined over 50 days. All runs of the model started with 10 aphids each in stages S_1 , S_2 , S_3 , S_4 and S_5 .

Results

Cages

Aphid populations persisted in cages without parasitoids for at least 13 weeks and were driven to extinction in all cages where parasitoids were present within 11 weeks (Figure 6). Populations of aphids exposed to 10-fold higher initial parasitoid densities were eliminated approximately 3 weeks sooner. Parasitoids successfully established in all cages where they were introduced, and were never observed in cages under the ‘control’ treatment. One cage from the ‘high’ treatment saw aphids return after being driven to apparent extinction, suggesting either undetected persistence or reintroduction during handling despite the precautions outlined above. Aphid populations in cages without parasitoids stabilized after approximately 8 weeks, with a mean aphid density between weeks 8 and 13 of 387 ± 24 aphids per plant.

Aphid pressure on plants (cumulative insect days) was significantly lower in ‘low-parasitoid’ cages than ‘control’ cages on week 7 ($CAD_{\text{control}} = 19,182$; $CAD_{\text{low}} =$

14,850; $t = 2.21$, d.f. = 14, $p = 0.044$) and all weeks thereafter. Cumulative aphid days were significantly lower in ‘high-parasitoid’ than ‘control’ cages starting immediately at the first observation 0.5 weeks after initiation ($CAD_{\text{control}} = 231$; $CAD_{\text{low}} = 191$; $t = 2.22$, d.f. = 14, $p = 0.043$) and continuing for all observations after.

Figure 7 shows mean aphid and parasitoid (unemerged mummies) densities over time for the two treatments where parasitoids were present. Parasitoid density peaked at week 7 and 8 when starting parasitoid density was low and at week 5 when parasitoid density was high. Proportions of winged aphids to wingless aphids grew over time within the cages, peaking at 3 weeks when starting parasitoid density was high, 7 weeks when starting parasitoid density was low, and leveling off after 8 weeks when parasitoids were absent (Figure 8). The proportion of fourth-instar nymphs plus adult aphids, the stages through which transgenerational fecundity compensation occurs, did not appreciably differ between treatments except after when aphid populations were already in decline (Figure 9).

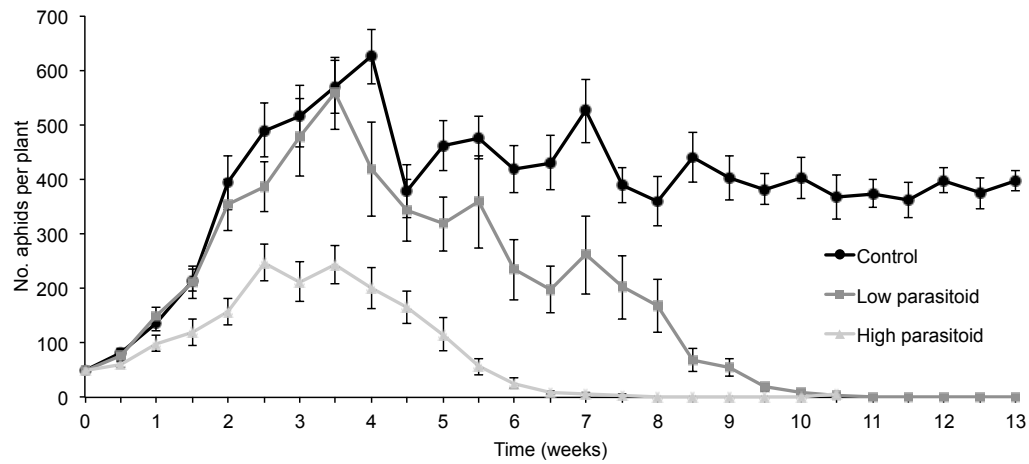


Figure 6. Mean (\pm SEM) number of *Aphis glycines* per plant in cages over time. ‘Control’ cages were free from parasitoids, while ‘low-’ and ‘high parasitoid’ treatments were initiated with 3 and 30 adult female *Lysiphlebus orientalis* wasps, respectively. All cages started with 50 *A. glycines* individuals per plant and six plants per cage.

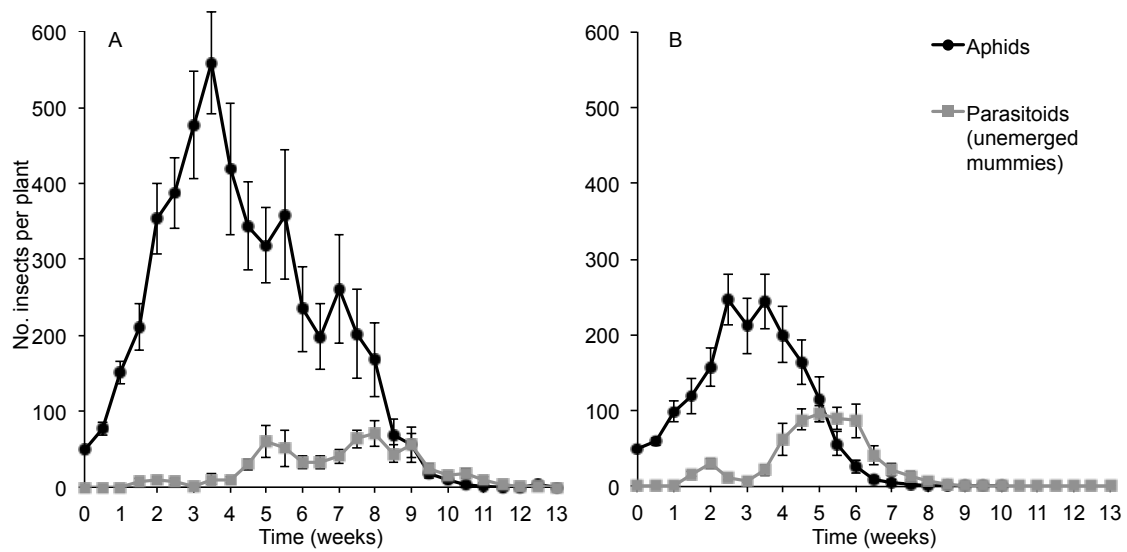


Figure 7. Mean (\pm SEM) number of *Aphis glycines* individuals and *Lysiphlebus orientalis* mummies (parasitoid pupae) per plant in cages over time. ‘Control’ cages were free from parasitoids, while ‘low-’ and ‘high parasitoid’ treatments were initiated with 3 and 30 adult female *L. orientalis* wasps, respectively. All cages started with 50 *A. glycines* individuals per plant and six plants per cage.

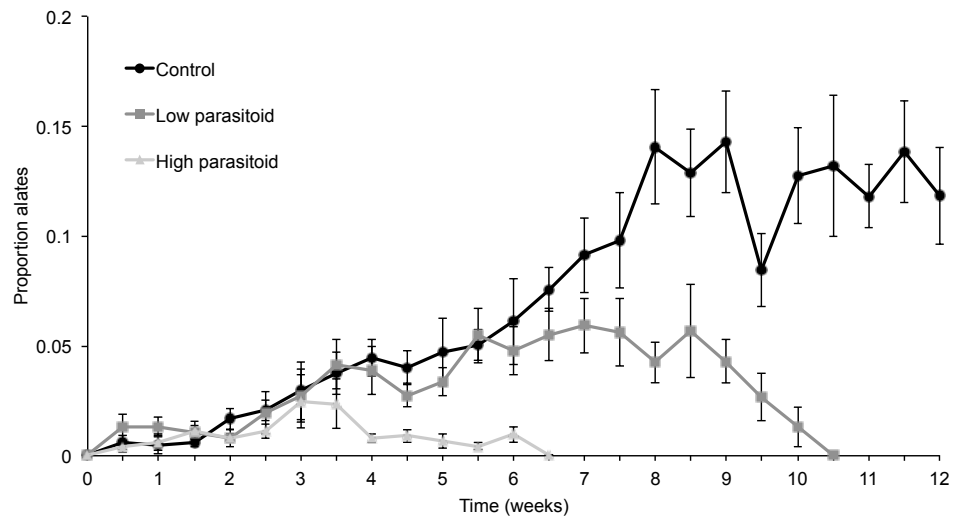


Figure 8. Mean (\pm SEM) proportion of winged (alate) *Aphis glycines* individuals per plant in cages over time. ‘Control’ cages were free from parasitoids, while ‘low-’ and ‘high parasitoid’ treatments were initiated with 3 and 30 adult female *Lysiphlebus orientalis* wasps, respectively. All cages started with 50 *A. glycines* individuals per plant and six plants per cage.

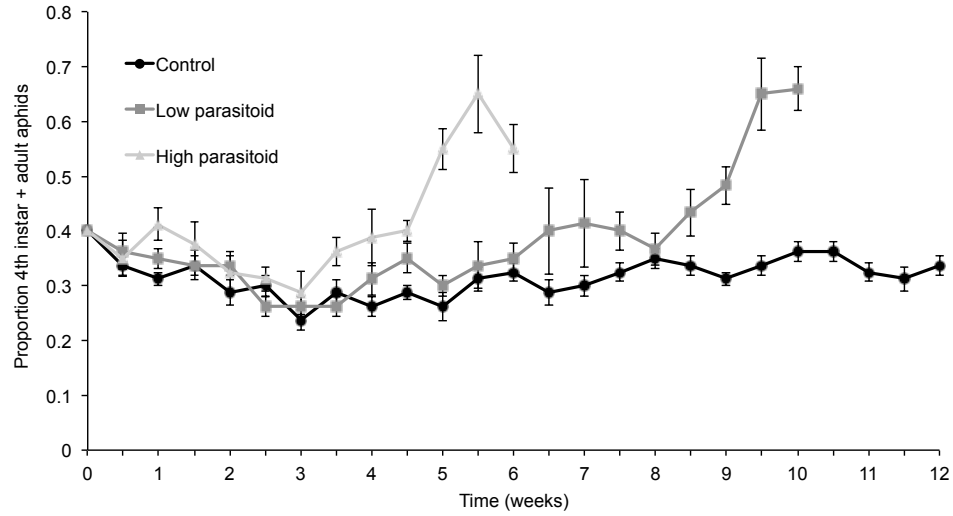


Figure 9. Mean (\pm SEM) proportion of fourth-instar plus adult *Aphis glycines* individuals per plant in cages over time. ‘Control’ cages were free from parasitoids, while ‘low-’ and ‘high parasitoid’ treatments were initiated with 3 and 30 adult female *Lysiphlebus orientalis* wasps, respectively. All cages started with 50 *A. glycines* individuals per plant and six plants per cage.

Model

Threshold parasitism pressures required to prevent aphid population growth for the four scenarios are highlighted in Table 2. When parasitized aphids were prevented from reproducing, threshold parasitism was lowest. When parasitized aphids could reproduce but their offspring were no different from the offspring of non-parasitized aphids, threshold parasitism pressure more than tripled. When transgenerational fecundity compensation was also included, threshold parasitism only increased slightly. Removing the demonstrated host stage preference of *L. orientalis* and replacing it with no preference between stages reduced the parasitism threshold by approximated one third. When background daily nymph survival was increased from 0.75 to 0.8, all parasitism thresholds increased by approximately 30% of their original value. Estimated aphid intrinsic growth rate, r , decreased as total percent parasitism increased, and decreased at the greatest rate when both post-parasitism reproduction and fecundity compensation were excluded (Figure 10).

Predicted aphid population densities initially oscillated then increased or decreased linearly on a logarithmic scale over time (Figure 11). Fecundity compensation resulted in the greatest aphid growth, slightly greater than under post-parasitism reproduction without compensation. When host-stage preference was removed but compensation maintained, the aphid population still grew but at a noticeably lower rate. Only when parasitism pressure was dramatically increased from 20% to 50% parasitism or host castration occurred did the aphid population decline.

The stage-structure of modeled aphid populations appeared qualitatively similar under the same range of scenarios other than host castration (Figure 12). First-instar nymphs were the most abundant in all cases (Figure 12). Under host castration (Figure 12A), second-instar nymph and adult aphids were approximately equally abundant at a level below that of first-instar nymphs, while third- and fourth-instar nymphs were least represented. However, under post-parasitism reproduction, non-preference by parasitoids, and increased parasitism pressure, second-instar nymphs were the second most abundant, followed by third-instar and adult aphids and finally fourth-instar nymphs (Figure 12B-E).

Table 2. Threshold parasitism pressure (y) and corresponding percent parasitism needed to prevent *Aphis glycines* population growth under four scenarios of attack by *Lysiphlebus orientalis*. Daily nymph survival includes all sources of nymph mortality other than parasitism. Daily non-parasitism adult mortality = 0.86 in all cases.

| <i>Scenario</i> | <u><i>Daily nymph survival = 0.75</i></u> | | <u><i>Daily nymph survival = 0.80</i></u> | |
|---|---|--------------|---|--------------|
| | y | % parasitism | y | % parasitism |
| <i>Host castration</i> | 0.63 | 12.6% | 0.84 | 16.8% |
| <i>Post-parasitism reproduction</i> | 1.95 | 39.0% | 2.53 | 50.6% |
| <i>Post-parasitism reproduction + transgenerational compensation</i> | 2.06 | 41.2% | 2.64 | 52.8% |
| <i>Post-parasitism reproduction + transgenerational compensation + even (no) stage preference</i> | 1.39 | 27.8% | 1.8 | 36.0% |

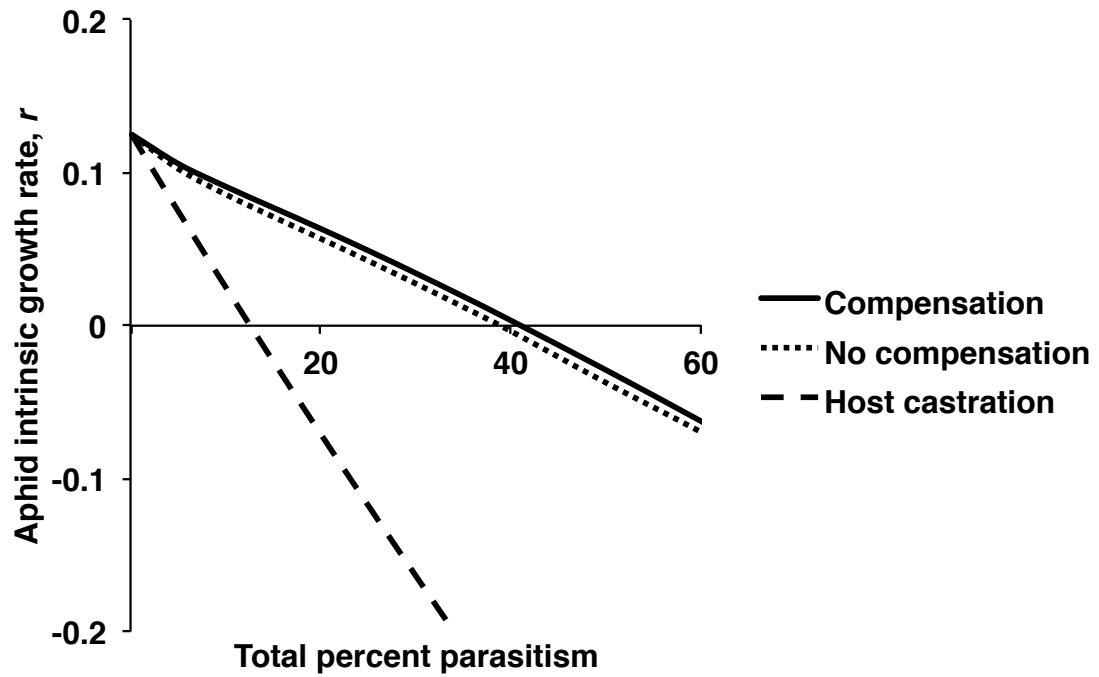


Figure 10. Estimated aphid intrinsic growth rate, r , as a function of total percent parasitism under three scenarios from the matrix model in Table 1. The solid line represents the case where both post-parasitism reproduction and transgenerational fecundity compensation occur. The dotted line represents the case where post-parasitism reproduction occurs while fecundity compensation does not. The dashed line represents the case where neither post-parasitism reproduction nor fecundity compensation occurs. Background daily nymph survival is 0.75.

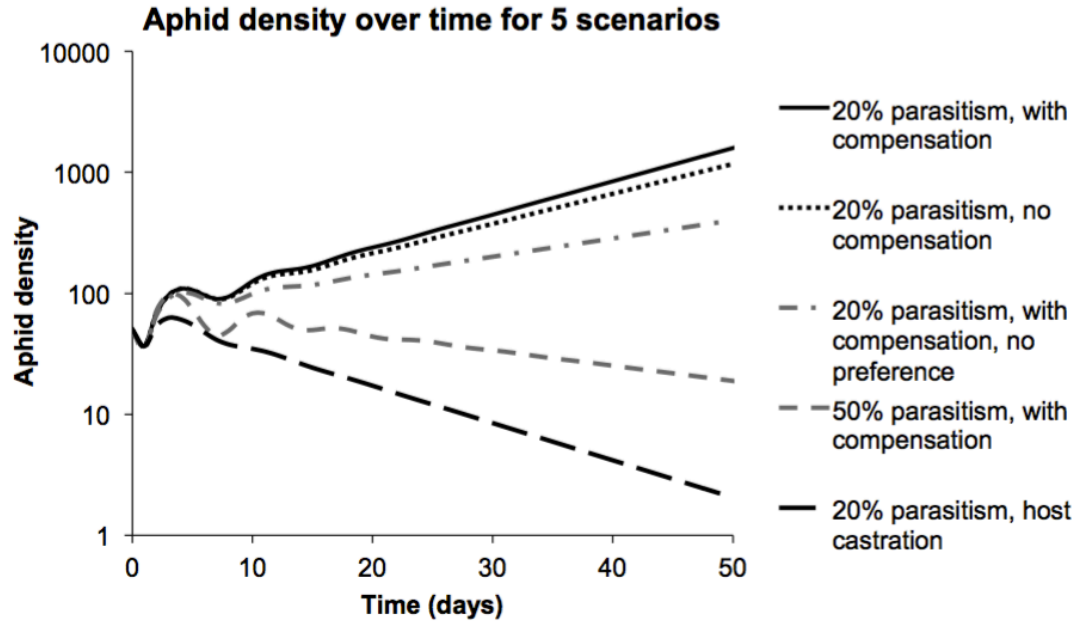


Figure 11. Predicted *Aphis glycines* density over time from the matrix model in Table 1 for five scenarios over 50 days. The solid black line represents the case where both post-parasitism reproduction and transgenerational fecundity compensation occur. The dotted black line represents the case where post-parasitism reproduction occurs while fecundity compensation does not. The dashed-dotted grey line represents the case where both post-parasitism reproduction and fecundity compensation occur, but the parasitoid shows no preference between host instars. The short-dashed grey line shows the case where both post-parasitism reproduction and fecundity compensation occur and percent parasitism is increased from 20% to 50%. Finally, the black dashed line represents the case where neither post-parasitism reproduction nor fecundity compensation occurs. Except for the grey dashed-dotted line, parasitoid host-stage preference is based on values shown in Figure 5. Background daily nymph survival in all cases is 0.75.

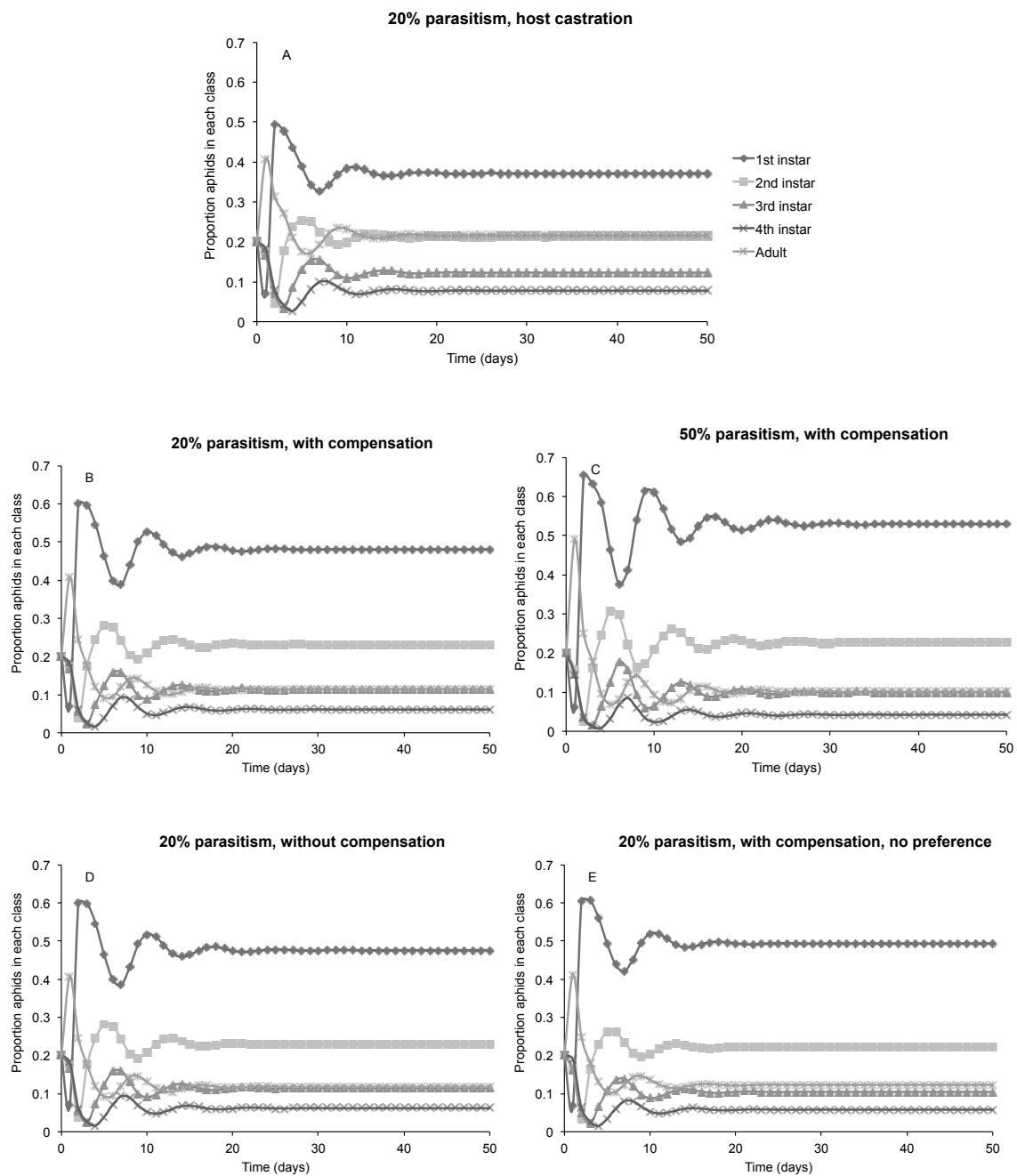


Figure 12. Predicted proportions of first-, second-, third-, and fourth-instar nymph and adult *Aphis glycines* individuals from the model in Table 1 over 50 days when (A) parasitized aphids cannot reproduce, (B) parasitized aphids can reproduce and exhibit transgenerational fecundity compensation, (C) parasitized aphids exhibit transgenerational fecundity compensation and parasitism pressure is increased from 20% to 50%, (D) parasitized aphids can reproduce but do not exhibit transgenerational compensation, and (E) parasitized aphids exhibit transgenerational fecundity compensation but parasitoid host-stage preference is removed. Except for (E), parasitoid host-stage preference is based on values shown in Figure 5. Background daily nymph survival in all cases is 0.75.

Discussion

All caged *A. glycines* populations exposed to either high or low initial parasitoid density were eventually driven to extinction, suggesting that transgenerational fecundity compensation in aphids cannot prevent suppression by parasitoids. Of the several traits of both aphids and parasitoids examined in the above matrix model, transgenerational fecundity compensation had the least pronounced effect on threshold parasitism pressure required to prevent aphid population growth. Aphid intrinsic growth rate, r , was very similar in cases with and without compensation (Figure 10). These findings, based on both caged and modeled populations, suggest that transgenerational fecundity compensation is not as impactful for aphid population growth as other aphid and parasitoid traits.

An interesting feature of the model is the large negative impacts of post-parasitism reproduction on threshold parasitism and aphid growth rate (Table 2, Figure 10). Many models of host-parasitoid interactions disregard post-parasitism reproduction by hosts (Godfray 1994, Murdoch et al. 2003). Furthermore, Lin and Ives (2003) found that post-parasitism reproduction in a similar aphid-parasitoid matrix model had minimal impact on aphid growth. However, *A. glycines* adults parasitized by *L. orientalis* in the present study only see a 32% reduction in fecundity on the third day of reproduction followed by the sharp cutoff on the fourth day (see Table 1), with no reduction on the second day. This contrasts with daily declines in fecundity of 73% and 92% on the second and third day of reproduction, respectively, for *A. glycines* adults parasitized by *A. colemani* (see Table 1 and Figure 5 in Lin and Ives 2003). Additionally, Lin and Ives

(2003) saw much lower post-parasitism reproduction by *A. glycines* adults parasitized by *A. colemani* than seen here with *L. orientalis*. The effect of post-parasitism reproduction may have also been exacerbated in this model due to the parasitoid's preference for older hosts. Ultimately, when parasitoid host-stage preference is removed from the model, threshold parasitism drops by one third. Even if transgenerational fecundity compensation does not have large impacts on aphid populations, accounting for post-parasitism reproduction in cases where it occurs may be quite important. Some early practitioners of biological control noted the apparent importance of parasitoid-induced sterility, or 'host-castration,' in hosts that are pests (Smith 1952, Drea 1968). Among host-parasite systems more broadly however, host sterility has been neglected as an important component of virulence until recently (Abbate et al. 2015). The ability of parasitoids to prevent reproduction in their hosts may be an attractive and often overlooked trait in biological control.

It is important to consider both safety and efficacy for control agents. While safety (low risk of impact on non-target species) is often evaluated in terms of host range (Babendreier et al. 2006, Barratt et al. 2010, 2016, Desneux et al. 2012, Acebes and Messing 2013, Raymond et al. 2016), introducing relatively 'safe' but ineffective agents still incur risk (Kaser and Heimpel 2015, Wajnberg et al. 2015, Kaser and Ode 2016). Within cages, at low parasitoid densities during the first six weeks, aphid pressure on plants did not differ from control. If suppression is delayed or dependent on high parasitoid density in the field, *L. orientalis* may not be a desirable control agent.

Aphids can be present in soybean fields starting in June in the North Central

region of the United States, but are typically not widespread until July, with highest densities and damage often occurring in August (Ragsdale et al. 2004, Hodgson et al. 2012). The caged populations here suggest that *L. orientalis* can cause *A. glycines* extinction after 11 weeks when starting at low density. If this timeline also applies to the field, *L. orientalis* would need to establish on *A. glycines* colonies within the first two weeks of aphid establishment in order to cause extinction before the end of August. More southerly growing regions within the aphid pest range may give the parasitoid a longer window of opportunity to suppress *A. glycines*. While *L. orientalis* was considered a specialist on *A. glycines* during initial research, it can attack other aphid species (Starý et al. 2010, Petrović et al. 2013). If *L. orientalis* is able to build its population on other aphid hosts and move onto *A. glycines* at higher initial densities, suppression may happen sooner as the high density parasitoid treatment saw extinction in 7 weeks.

While often used, cages are inherently unrealistic representations of the real world due to restricted foraging and dispersal by parasitoids, elevated recolonization by winged aphids attempting to emigrate, and elevated temperature and humidity (Luck et al. 1988, Kindlmann and Dixon 2010). Also, while the matrix model used includes a large number of parameters based on real traits, it does not explicitly account for parasitoid population growth or resource competition between aphids, which certainly change through time. Regarding competition between hosts, Cameron et al. (2007) showed that competition between parasitized and non-parasitized individuals may negatively affect host populations. It is also worth noting that caged aphid populations consisted of higher

proportions of fourth-instar and adult aphids than the model predicts (Figures 9, 12), showing that the model may not be an ideal representation of the caged populations. However, I consider these two methods (caged populations and matrix models) as complementary tools that can still be useful when attempting to evaluate imported classical biological agents prior to release, and to determine whether compensatory responses by hosts can disrupt their suppression.

**3. Generalization of transgenerational fecundity compensation:
demonstration in the aphid *Aphis craccivora* in response to
parasitism by two competing parasitoids**

Introduction

Parasitoid-induced transgenerational fecundity compensation, as described in Chapter 1, is a phenomenon where daughters of parasitized aphids reproduce at a greater rate than the daughters of healthy aphids. The first description of this phenomenon found in Chapter 1 is in soybean aphids (*Aphis glycines*) under attack by the parasitoid *Lysiphlebus orientalis*. A major question posed by that work is whether transgenerational fecundity compensation is unique to that particular aphid-parasitoid association or can be found in other species.

At the end of Chapter 1, I suggest three hypotheses for the origins of transgenerational fecundity compensation. First, it may be the result of a parasitized mother aphid actively increasing investment in her remaining offspring, which should be adaptive since aphid lineages with compensation should be more fit (Leventhal et al. 2014). Second, it may be an adaptive and ‘intentional’ manipulation of aphid physiology by the parasitoid. This would be likely to evolve under close host-parasitoid coevolution, where parasitoids that induce compensation should increase the likelihood of there being more hosts available for future generations (Dubois et al. 2013). Finally, it may simply have originated and occur as a side effect of parasitism. This could be caused by the interactions of parasitoid venom, larvae, and teratocytes with aphid embryos and nutritional endosymbionts (Dahlman and Vinson 1993, Cloutier and Douglas 2003, Pennacchio and Mancini 2012, Burke and Strand 2014).

Demonstrating transgenerational fecundity compensation in other aphid-parasitoid species associations could give support for one more of the hypotheses above. If

compensation occurs when a new aphid species is attacked by *L. orientalis* but not other parasitoids, that would suggest that this strategy of host manipulation may have uniquely evolved in *L. orientalis* to increase host abundance for future generations. If instead compensation occurs in this new aphid when it is attacked by both *L. orientalis* and another parasitoid, that would show this phenomenon is not unique to either *A. glycines* or *L. orientalis*, and is more likely to be a neutral effect or evolved by the aphid hosts. Finally, if compensation does not occur in this new aphid, then it may be due to something unique about association of *A. glycines* and *L. orientalis* and their coevolutionary history in East Asia. Compensation may also in this case be the result of or something unique about the laboratory conditions where the research in Chapter 1 took place, or the product of extended lab rearing of *L. orientalis* on *A. glycines* (Starý et al. 2010).

In this chapter, I used similar methods as in Chapter 1 to establish whether transgenerational fecundity compensation occurs in the cowpea aphid (*Aphis craccivora* Koch) (Hemiptera: Aphididae) when attacked by both *L. orientalis* and *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae). I also measured the host-stage preference for each of these parasitoids on *A. craccivora*, both to compare with the preference observed in Chapter 1 and because preference may have strong impacts on host suppression as shown in Chapter 2. *Lysiphlebus fabarum* has long been established in Europe and is thought to have a long coevolutionary history with European populations of *A. craccivora* (Petrović et al. 2013, 2015, Starý et al. 2014). *Lysiphlebus orientalis* however is thought to have only recently arrived in Europe from its native

range in East Asia (Starý et al. 2010, Petrović et al. 2013, 2015). While the two parasitoid species are congeners and were initially confused in European collections before *L. orientalis* was formally described, they are members of two phylogenetically distinct species groups (Starý et al. 2010, Petrović et al. 2013, 2015). While *Lysiphlebus orientalis* is known as an exclusively asexual (thelytokous) species (Starý et al. 2010, Petrović et al. 2015), *L. fabarum* exists in both sexual and asexual populations (Engelstädter et al. 2011, Sandrock et al. 2011, Ameri et al. 2015, Petrović et al. 2015). Only the asexual form of *L. fabarum* was collected and used in the present work.

Methods

Insects

Aphis craccivora were collected from wild-growing *Medicago sativa* L. (Fabacea) (alfalfa) plants in Belgrade, Serbia to be used as hosts for experiments. A colony was started from approximately 80 individuals collected on several neighboring plants, which were reared for at least one generation on potted and caged *M. sativa* plants in a greenhouse (approximately at $25\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ and L14:D10 h photoperiod) prior to any of the below experiments. Any aphids that mummified due to being parasitized before collection were promptly removed from the colony and destroyed.

Aphis fabae Scopoli (Hemiptera: Aphididae) (black bean aphids) were collected from *Chenopodium album* L. (Amaranthacea) (lamb's quarters) plants in Zemun, Serbia to be used as hosts for rearing parasitoids. A colony was established from approximately 200 individuals collected from a small group of neighboring plants in a residential

boulevard. As with *A. craccivora*, this colony was reared for several generations on potted and caged *C. album* plants in a greenhouse (conditions same as above) and checked to ensure no parasitoids were present.

Lysiphlebus fabarum and *L. orientalis* were both collected from mummies within *Aphis fabae* colonies on *C. album* in Zemun, Serbia. Live newly emerged adult wasps were identified to morphospecies under a dissecting microscope and released into cages containing *Aphis fabae* on *C. album*. The primary morphological trait used to distinguish *L. fabarum* from *L. orientalis* was the length of the forewing metacarpus, also known as the R1 vein (reaching the apex in *L. fabarum* and much shorter in *L. orientalis*).

Mummies were collected from a location known to support populations of both species (Petrović et al. 2013). Once parasitoid colonies were established, approximately 5 adult parasitoids were removed from each cage each week, killed in 95% EtOH, and examined under a dissecting microscope to confirm identification. Both of these endoparasitoids are solitary koinobionts, so the aphids continue to develop temporarily after parasitism and a single aphid individual can only support the development of one parasitoid individual (Godfray 1994).

Compensation

Single third- and fourth-instar and newly molted (< 24 h) adult apterous *A. craccivora* were individually transferred with a fine paintbrush from potted source plants to cuttings of inverted *M. sativa* leaves. First- and second-instar aphids were not included because prior work with *Aphis glycines* indicated that aphids parasitized at these stages did not reproduce (see Chapter 1, also see Lin and Ives 2003). Aphids were allowed to

settle for 5 min and then one newly emerged adult female *L. orientalis* or *L. fabarum* was introduced near an aphid using a fine paintbrush, and both were covered with a 6-mm-wide clear plastic observation dome. After a successful ‘sting’ was observed under a dissection microscope (described below under “Preference” and in Chapter 1), the wasp was removed and the aphid was transferred to a single 25-cm cutting of *M. sativa* set in water in a 200-ml clear plastic cup. A 250-ml clear plastic cup with the bottom removed was inverted and placed over smaller cup holding the cutting. The top was then covered with no-see-um mesh secured by a rubber band to ensure aphid containment. Nine third-instar, 11 fourth-instar, and 11 adult aphids were allowed to be stung by *L. fabarum*. Seven third-instar, 9 fourth-instar, and 9 adult aphids were allowed to be stung by *L. orientalis*. As a control group for comparison, an additional 10 third-instar, 10 fourth-instar, and 10 adult aphids were similarly handled but not exposed to parasitoids. Other than four aphids that died (not included in the replicates reported above), all aphids observed as ‘stung’ above mummified.

Aphid reproductive state and fecundity were observed daily until aphids mummified. The 1st day that new nymphs were observed, a single nymph from this cohort was transferred to a new *M. sativa* cutting for daily observations. This second generation included 10, 8, and 9 aphids whose parents were stung by *L. fabarum* or *L. orientalis* or not stung in the fourth stadium, as well as 10, 8, and 10 aphids whose parents were stung by *L. fabarum* or *L. orientalis* or not stung as adults, respectively. A total of eight aphids from this second generation were lost or died, which is reflected in the replicates. We continued to observe each of these two generations of aphids from the

three lineages for at least 4 days after reproductive maturity. All aphids in the experiment were housed in the same climate-controlled rearing room (approximately at $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and L16:D8 h photoperiod), with treatments and generations interspersed across bench space and time. We compared the numbers of nymphs produced by aphids in both lineages each day, with the developmental stage and stung status of the original parent as factors in two-way ANOVA and with P-values adjusted using Bonferroni corrections in R v. 3.0.0 (R Core Team, 2013). We also pooled reproduction across the 4 days to determine total effect of the two factors on nymph production. Residuals were examined to ensure that model assumptions were met.

Preference

I conducted choice tests of parasitoid preference for aphid stage in a manner similar what was described in Chapter 1 for *L. orientalis* and *A. glycines*, and similar to what was performed by Wyckhuys et al. (2008). Newly emerged adult female *L. orientalis* or *L. fabarum* were presented with 25 apterous aphids (five each in the first, second, third, and fourth stadium, and adults), which were allowed to settle for 5 min on a collection of inverted excised *M. sativa* leaves prior to introduction of the wasp. We monitored wasp behavior by recording ‘encounters,’ ‘attacks,’ and ‘stings’ over a 5-min period, as defined in Chapter 1 for *L. orientalis*. Although observed stings do not guarantee oviposition or parasitism, no aphids observed as stung in the compensation experiment described above (other than the four aphids that died) failed to mummify. A total of 15 *L. orientalis* and 15 *L. fabarum* individuals were assessed, with leaves and

aphids being replaced prior to each assessment. Aphid defensive behaviors were not scored.

As in Chapter 1, analysis of preference was modified from Weisser (1994) and Wyckhuys et al. (2008), using Manly's beta statistic (Manly 1974). Preference was scored as the deviation in the number of individual aphids stung from the number of aphids encountered by calculating

$$\beta_j = \frac{(x_j/A_j)}{\sum_{i=1}^5 x_i/A_i},$$

where x_i is the number of stings and A_i is the total number of encounters recorded for aphids in growth stage i , and x_j is the number of stings and A_j is the total number of encounters for aphids in growth stage j , with five growth stages considered (i.e., $j = 1, 2, 3, 4, 5$). If β_j is equal to $1/5$ for all stages, the parasitoid shows no preference, but if β_j is significantly greater than $1/5$ for any given stage, the parasitoid prefers that stage. This formulation of Manly's beta, which allows for repeated behaviors, was chosen because parasitoids of both species were observed encountering and stinging individual aphids multiple times in the same trial. Manly's beta values were compared using non-parametric Kruskal-Wallis tests, followed by Nemenyi pairwise tests for multiple comparisons of mean rank sums from the PMCMR package in R (R Core Team 2013, Pohlert 2014).

Results

Compensation

Only two aphids stung in the third stadium by *L. fabarum* out of nine became reproductive, and in both cases only one nymph was laid (Figure 13). Fourth-instar and adult *Aphis craccivora* stung by both *L. orientalis* and *L. fabarum* successfully reproduced for up to 4 days (Figure 13). Parasitism significantly reduced aphid fecundity, primarily due to significant decreases on days 3 and 4 (ANOVA, all days pooled: $F_{2,53} = 11.16$, $P < 0.001$; day 1: $F_{2,53} = 0.310$, corrected $P = 1.0$; day 2: $F_{2,53} = 0.366$, corrected $P = 1.0$; day 3: $F_{2,53} = 5.788$, corrected $P = 0.002$; day 4: $F_{2,53} = 14.96$, corrected $P < 0.001$). There was no significant effect of aphid stage on overall reproduction over the first 4 days for parasitized aphids (all days pooled: $F_{1,53} = 0.139$, $P = 0.710$; each day 1-4 separately: corrected $P = 1.0$). Among the next generation of aphids, there was also no significant effect of the mother's stage when stung (all days pooled: $F_{1,49} = 0.130$, $P = 0.885$; each day 1-4 separately: corrected $P = 1.0$). However, there was a significant effect of mothers' parasitism on reproduction (all days pooled: $F_{2,49} = 15.51$, $P < 0.001$), with offspring of parasitized aphids reproducing at a greater rate than offspring of not stung aphids (Figure 14). The increase was equal to approximately one additional nymph produced per day by the offspring of parasitized compared to non-parasitized aphids, though there were no significant differences between treatments by day once P-values were corrected (day 1: $F_{2,49} = 4.283$, corrected $P = 0.077$; day 2: $F_{2,49} = 0.639$, corrected $P = 1.0$; day 3: $F_{2,49} = 4.485$, corrected $P = 0.065$; day 4: $F_{2,49} = 3.51$, corrected $P = 0.15$).

Preference

Aphis craccivora growth stage had a significant effect on oviposition preference by both adult female *L. fabarum* (Kruskal-Wallis: $\chi^2 = 11.78$, d.f. = 4, $P = 0.019$) and *L. orientalis* (Kruskal-Wallis: $\chi^2 = 12.01$, d.f. = 4, $P = 0.017$) wasps. For *L. fabarum*, the only significant pairwise difference in preference was between third-instar and first-instar nymphs ($P = 0.021$), with third-instar nymphs preferred (Figure 15A). For *L. orientalis*, the only significant pairwise difference in preference was between fourth-instar and first-instar nymphs ($P = 0.017$), with fourth-instar nymphs preferred (Figure 15B).

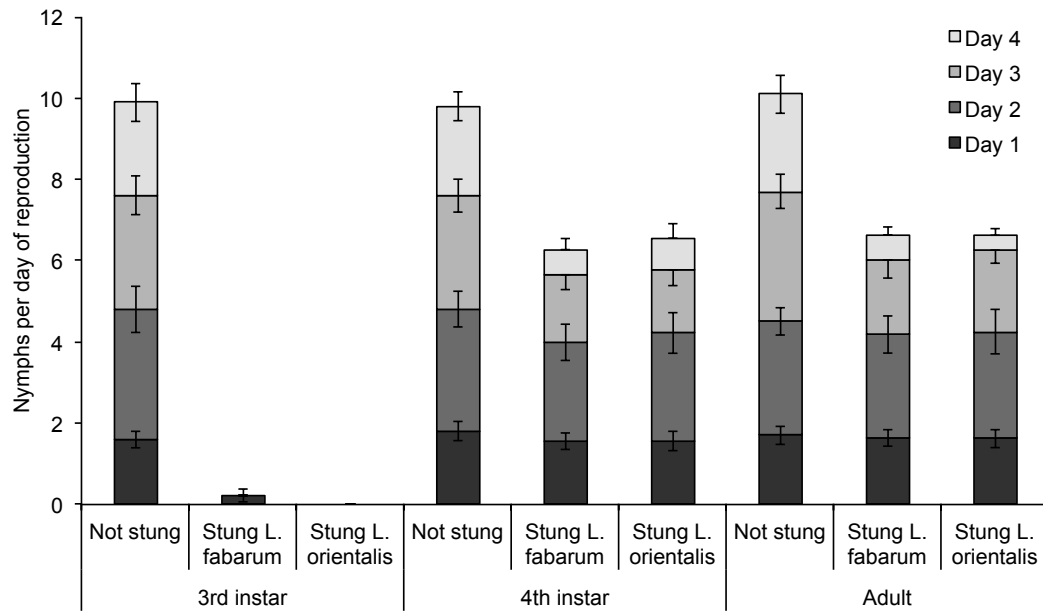


Figure 13. Mean (\pm SEM) daily reproduction by *Aphis craccivora* aphids after being stung or not stung by *Lysiphlebus orientalis* or *Lysiphlebus fabarum* as third or fourth instar, or as newly molted adult. ‘Days’ are days of reproduction, with ‘day 1’ being the first day nymphs were observed. Each bar represents the mean number of nymphs laid, separated by day.

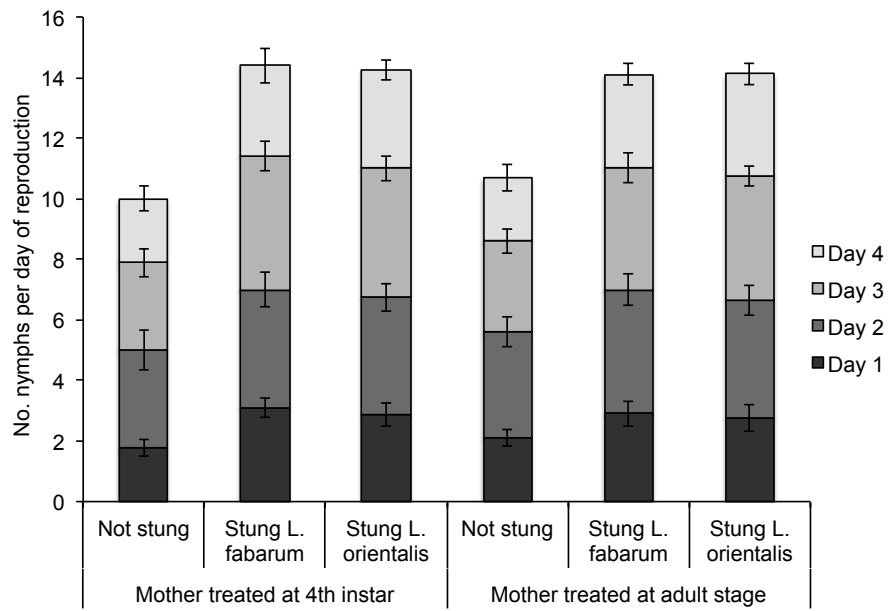


Figure 14. Mean (\pm SEM) daily fecundity of *Aphis glycines* aphids whose mothers were either stung or not stung by *Lysiphlebus orientalis* or *Lysiphlebus fabarum* as fourth instar or adult. ‘Days’ are days of reproduction, with ‘day 1’ being the first day nymphs were observed. Each bar represents the mean number of nymphs laid, separated by day.

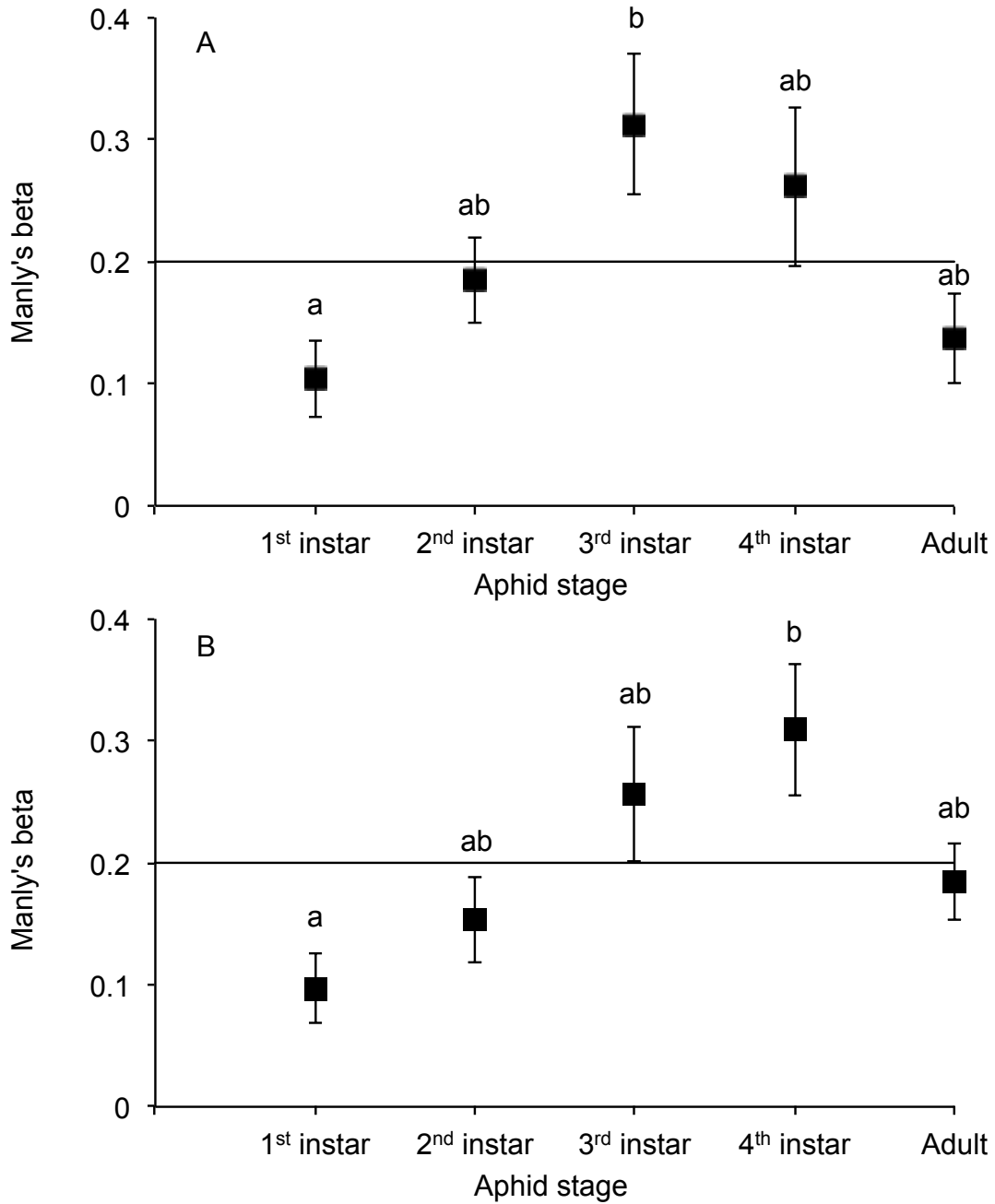


Figure 15. Mean (\pm SEM) host stage preference of (A) *Lysiphlebus fabarum* and (B) *Lysiphlebus orientalis* given encounter rate. The horizontal line corresponds to Manly's β value (0.2) for no preference between stages. Values above the line suggest preference. Means capped with different letters are significantly different (Nemenyi pairwise comparisons: $P < 0.05$).

Discussion

Through this work I have demonstrated that transgenerational fecundity compensation occurs in a second aphid host, *Aphis craccivora*, when attacked by both the recently introduced parasitoid *Lysiphlebus orientalis* and its native congener *L. fabarum*. An increase in reproductive rate of approximately one nymph per day over the first four days of reproduction was observed for daughters of aphids parasitized by either parasitoid, an effect similar in magnitude to what was reported for *A. glycines* under attack by *L. orientalis*. *L. orientalis* tended to prefer slightly older aphids than *L. fabarum*, though both accepted all aphid growth stages as hosts.

Finding transgenerational fecundity compensation in two additional aphid-parasitoid associations suggests that this newly described phenomenon may be general among aphids and their parasitoids. It also suggests that this form of compensation is either under control of the aphid hosts or a neutral effect of parasitism, and, since European *A. craccivora* and Asian *L. orientalis* form a relatively new association, did not evolve as an adaptive manipulation of hosts by tightly coevolving aphids and parasitoids (Petrović et al. 2013, Dubois et al. 2013). It is worth noting that both aphid species and both parasitoid species where transgenerational fecundity compensation has been demonstrated are congeners, which leaves open the possibility that it may be restricted in to the genera *Aphis* and *Lysiphlebus*. Additionally, all populations of *L. orientalis* and *L. fabarum* studied here and in Chapter 1 reproduce asexually. It is not clear what effect sexual reproduction may have on this phenomenon.

Among the two parasitoids in this study, *Lysiphlebus orientalis* has only recently

been found in Europe and is presently restricted in distribution to central and northern Serbia (Petrović et al. 2013, 2015). While transgenerational fecundity compensation was proposed as a potential driver for *L. orientalis* to expand its range and potentially displace and disrupt *L. fabarum* (Chapter 1, Petrović et al. 2013), this seems like less of a concern now that compensation has been demonstrated in *L. fabarum* as well. However, *L. orientalis*' preference for slightly older aphids than *L. fabarum* may still have population level consequences, since only more mature aphids are able to reproduce after being parasitized. Resource preemption through host-stage use has been shown to be sufficient to drive competitive displacement in parasitoids (Murdoch et al. 1996). If *L. fabarum* is accepting a wider range of host stages and parasitizing younger aphids than *L. orientalis* is, *L. fabarum* may have a competitive advantage. However, spatial segregation may allow for coexistence even when one competitor is superior (Borer et al. 2004). *Lysiphlebus fabarum* and *L. orientalis* have been collected from the same hosts in the same geographic area (Petrović et al. 2013), but may not have perfectly overlapping host ranges or habitat use strategies.

Interspecific larval competition by these two parasitoids where their geographic and host ranges overlap will also affect the outcome of interaction between the two species, and *Lysiphlebus* spp. are known to compete within super- and multi-parasitized hosts. For instance, *Lysiphlebus testaceipes* larvae have been shown to successfully compete against other aphid parasitoids in the genera *Aphidius* and *Lipolexis*, though the outcome of competition is often dependent on relative age of the larvae (Völkl and Stadler 1991, Persad and Hoy 2003, Sampaio et al. 2006). The competitive ability of the

larvae of these two species and how they interact within a shared host is unknown.

The aphid in this study, *Aphis craccivora*, is known to harbor secondary defensive bacterial endosymbionts which protect against some aphid parasitoids (Brady and White 2013, Brady et al. 2014,). These symbionts can act quite specifically (Brady and White 2013, Asplen et al. 2014, Mclean and Godfray 2015) and can also act as drivers of host-race formation (Rouchet and Vorburger 2014). Furthermore, parasitoids have been shown to be capable of transmitting these defensive symbionts between aphids (Gehrer and Vorburger 2012). Unfortunately I was unable to determine whether the aphids used in the experiments above were harboring any secondary endosymbionts. However, the success with which both parasitoid species parasitized these aphids suggests secondary endosymbionts were either not present or not effective. Additionally, aphid defensive symbionts may be easily lost from lab-reared colonies (Dykstra et al. 2014). Future work investigating fecundity compensation in other aphid and parasitoid species as well as competition between parasitoids, especially when insect populations are recently collected from the field, should confirm the presence or absence of defensive aphid endosymbionts to ensure real effects are not masked.

Conclusions

Through laboratory experiments, I have described a novel phenomenon where offspring of parasitized aphids reproduce at a greater rate than offspring of non-parasitized aphids, which I have named ‘transgenerational fecundity compensation.’ This is, to the best of my knowledge, the first description of this kind of interaction between an animal and its parasite. I have proposed a likely mechanism for this form of fecundity compensation in aphids based on the literature and dissections of live aphids. I have also demonstrated through both caged populations and modeling that transgenerational fecundity compensation may have relatively minor effects on host population dynamics. Finally, I have shown that transgenerational fecundity compensation occurs in two additional aphid-parasitoid associations, suggesting it may be a general phenomenon among aphids and their parasitoids and not restricted to the association where it was first described. It remains to be shown whether transgenerational fecundity compensation occurs in any other host-parasitoid or host-parasite systems beyond aphids, but this work can serve as a reference for the kinds of straightforward experiments that may be used to demonstrate this phenomenon and its effects.

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